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ELECTRIC SHOCK *

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The English, American, German, and French literature contains many isolated reports and abstracts of accidents caused by electricity. Great emphasis is placed on the bizarre effects and playful vagaries of electrical current, less is said about treatment and least about prevention.

LITERATURE

No attempt will be made to give a complete review of the literature dealing with electric shock; that is already voluminous and has been clearly described in a recent paper by Jaffé,[1] who in addition gives an excellent bibliography.

The macroscopic and microscopic observations on the nervous system in death from electric shock vary greatly. Some authors found no changes, others, lesions only when large amounts of electricity passed through the body for a long period of time. Some described small perivascular hemorrhages especially in the medulla oblongata and floor of the fourth ventricle, while others reported marked edema of the brain.

Spitzka [2] in his study of the brain of electrocuted animals found no apparent change in the nerve cells, but described areas of disruption and destruction with capillary hemorrhages. In the pons, medulla and cord curious circular areas were encountered with rarefied centers and peripheral zones of condensation, which he suggested resulted from the electrolytic liberation of gas in perivascular spaces.

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Werner [3] in his article dealing with electrocution as a judicial homicidal agent described the brain at autopsy as being very hot and recorded a temperature in the brain of 145° F. Jaffé feels that this excessive heat offers a sufficient explanation for the vacuolization seen within the brain.

Corradi [4] found chromatolysis of ganglion cells in the nervous system of dogs killed by electricity. Mott and Schuster,[5] in addition to perivascular hemorrhages and chromatolysis of ganglion cells in cortex and medulla, described a peculiar intracellular and intranuclear network. Capogrossi [6] also observed marked variation and destruction of the Nissl bodies. Kawamura [7] described shrinkage and hyaline changes in the nerve cells, and fragmentation of the tigroid bodies within the medulla. Gubler [8] reported a vacuolization of the nerve cells and Jellinek [9] destruction of the cells with extrusion of the nuclei.

The histologic changes in the peripheral nerves have been studied by Bolognesi [10] and Pietrusky;[11] the former found swelling and twisting of the axones, and breaking down of the nerve sheaths, while Pietrusky described a fusing together of the nuclei of the sheaths of Schwann.

Report of Experimental Work

A rather small series of animals, namely, one dog and one cat, and twenty guinea pigs, were used in the study of the histologic changes following electrocution.

These animals were divided into two groups; Group I including the dog and the cat, in which death followed immediately after a single shock, and Group II comprising the guinea pigs in which one to three sublethal shocks were given daily over varying periods of time. Of this second group the first animal was shocked on one day only, the second was shocked for two successive days, the third for three days and so on; the animal receiving the greatest number of shocks was shocked every day for nine successive days. Besides varying the number of shocks for each animal, the interval of time that elapsed after the shocking and before the animals were killed and autopsied was also varied; and this ranged from eighteen hours to fourteen days.

The guinea pig was selected because it is a fairly reliable laboratory animal and especially because it is small. When a large animal

is severely shocked the heart stops during the period the current is being applied and then goes into ventricular fibrillation, but seldom, if ever, recovers. The guinea pig heart behaves in much the same manner but if the shock has not been too prolonged the heart recovers its normal rhythm and the animal lives. Briefly, it is considered that the impulse or impulses that are traveling through the myocardium while the ventricle is fibrillating have relatively fewer excitable muscle groups to stimulate in a smaller heart, and consequently fibrillation is of shorter duration and recovery therefore more probable.

In shocking the guinea pigs the current from a six volt battery was passed through a small induction coil, and the strength of the induced current was varied by adjusting the central core. Tissue from a human came to us from a case of legal electrocution, while the dog and cat were obtained immediately after death following electrocution by the Animal Rescue League, and in this case a current of 110 volts was used.

The electrodes that were applied to the guinea pigs were two bared wires; one was passed through and across the mouth, the other was applied to one leg moistened with saline. The Animal Rescue League used a specially built copper cage and collar. In either case excellent contacts were obtained.

Preliminary to shocking the animals sufficient ether was given to quiet them and to permit fastening of the electrodes. Two of four control animals were given ether by inhalation in much larger amounts than the test animals and over a longer period of time.

As soon as the current was passed through the body there was a general contraction of all the muscles, sometimes accompanied by a shriek, and the animal maintained this rigid tonic state as long as the current was applied. The recovery after the shock varied; when the shock was of short duration and not severe, recovery was nearly instantaneous. On the other hand, with more prolonged shocking and with a stronger current, or in animals which had been shocked on several occasions, the recovery was slower, the animals appeared weaker, tremulous and had difficulty in supporting themselves. In several cases they completely relaxed for several minutes, seemed stuporous and assumed unusual catatonic positions. For hours after the shock many of the animals were unusually nervous and excitable, and in two cases they refused ordinary diet but chewed paper con-

tinually for hours and hours. When a fatal shock was given, the animals remained rigid for several seconds after the current was removed, then slowly passed into complete relaxation.

The current probably plays its greatest part in striking the central nervous system and the many altered physiologic signs which become manifest in the different systems appear to be simply visible expressions of extreme stimulation or marked alteration within the central nervous system.

While the current is passing through the body the animal is rigid, the heart stops beating and respirations cease; and eversion of the anus, defecation, micturition and emission occur almost regularly. Occasionally the animals vomit at the onset, and in two there was a prolapse of the rectum which returned as soon as the current was removed. Certain deranged physiologic signs that appear in the nervous system, the heart and blood vessels and in the respiratory system are unusually interesting and bear a more detailed description. Furthermore, to determine whether defecation, micturition, emission and prolapse of the rectum were merely passive reactions secondary to contraction of the abdominal muscles, or result from the stimulation and response of those viscera independently, we repeated the experiments in another group of guinea pigs with the abdominal cavity opened and with still another group in which the animals had received atropine.

The Immediate Response of the Heart to Electric Shock

The heart was examined in each test by auscultation before and immediately after the current was applied, but during the period of shock the sounds of contracting skeletal muscle were so loud it was impossible to follow the cardiac sounds, and consequently it was necessary to observe the heart's action directly.

With the animal lightly under ether and with the heart exposed, an electric shock of five seconds duration was given in the usual manner. As soon as the current was applied the heart contracted strongly and remained in systole except for two to three irregular beats which probably represent vagus escape. During the entire period of shock and for two to three seconds after, the ventricles showed an almost invisible and constant tremor. This was followed by a definite fibrillation of the ventricles which lasted three to five

seconds and this in turn was succeeded by irregular rather forceful contractions which continued for nearly ten seconds. The rhythm eventually became more regular but the rate was slower. This slowing of the heart was seen only in the animals whose hearts had been exposed, whereas in the unoperated animal which recovered after the shock the heart rate was temporarily greatly increased.

This simple experiment was of interest because we were able to follow the heart simultaneously and by auscultation which enabled us to interpret more clearly the irregularities noted in the animals from day to day.

The Immediate Response of the Heart to Electric Shock after Blocking the Vagus with Atropine

In each of these experiments $\frac{1}{100}$ to $\frac{1}{150}$ gr. of atropine dissolved in 5 cc. of sterile water was slowly injected into the leg veins of large healthy guinea pigs. With the animals lightly under ether, they remained sufficiently quiet to permit constant auscultation of the heart. After about ten to fifteen minutes the heart rate had nearly doubled but retained its normal regularity.

In the first of these experiments the heart was not exposed, so we could not follow the beats while the current was passing through the body, but when the current was shut off there was no pause or irregularity and the heart continued its rapid but regular course.

When the heart was exposed and the same experiment repeated, we watched the heart continue beating while the current was passing through the body and we observed no change in rate; neither was there a pause nor irregularity after the current ceased. This was important for it showed that when the vagus was blocked with atropine, an electric shock, which in a control animal was sufficient to stop the heart, produce ventricular fibrillation, irregularity and acceleration, effected no appreciable response in either rate or rhythm.

The Delayed Response of the Heart following Repeated Shocks

In the course of the experiments it was observed that there was a definite change in the heart which became more marked with each additional shock. When the animal was first shocked it usually made

a good recovery and in a minute or two appeared quite normal, but after shocking an animal for several days less current was required to produce the same effect and the recovery was much slower. In such a case the cardiac irregularity was more easily induced and the irregularity and acceleration lasted several times longer than that following the shocking of a normal animal. Furthermore, great caution had to be exercised in shocking animals that had been shocked previously on several occasions lest they suddenly die. Three examples from our series will make this point clearer. One of the guinea pigs died on the fifth day immediately after five shocks; another on the sixth immediately after six shocks. The third, apparently quite well and with a normal cardiac rate and rhythm, had been given seven daily shocks. The heart of the latter pig was being examined preliminary to the eighth shock when the animal exerted a little effort and suddenly died. In the case of the first two we had used less current and for a shorter period than on the preceding day. The manner of death in all three cases was the same. After the heart first stopped the cardiac sounds were never again audible except for three or four irregular and indistinct beats. The animals lay completely relaxed while the respirations continued for two to three minutes in a slow irregular gasping manner.

These observations indicate that as a result of electric shocks there is a profound disturbance in the cardiac mechanism which may be largely obscured by the presence of a normal rate and rhythm.

The Response of the Blood Vessels to Shock

There was a change in the peripheral blood vessels following shock which if duplicated by the blood vessels of the central nervous system might have some consequent bearing on the lesions that later appear in the nerve tissue. By placing the ear of a guinea pig before a light the smaller vessels stood out quite clearly, and these were moderately accentuated if the ear was lightly rubbed. During the shock no apparent change was seen in this rich vascular network, but, about thirty seconds after the current was withdrawn, there was a gradual constriction of the vessels and the ear became abnormally blanched and remained anemic for five to ten minutes. The delayed vasoconstriction resulting from sympathetic stimulation simulates the vascular phenomenon in the condition clinically

spoken of as "surgical shock," and in itself would be sufficient to produce a moderate degree of ischemia with resulting oxygen deficiency in the areas involved.

The Response of the Abdominal Viscera to Shock

We have previously mentioned the observations seen in the normal animal which has neither been operated upon nor received atropine, namely, defecation, micturition and emission and occasionally prolapse of the rectum and vomiting. When the current was applied after the peritoneal cavity had been widely opened to remove the factor of contracting skeletal muscle, we were able to observe the contracting seminal vesicles with the resulting emission, and a slow feeble contraction of the bladder and intestines, although in the latter there was no apparent change in the peristaltic movements.

The pressure within the peritoneal cavity was greatly increased during shock as a result of the contraction of the diaphragm and skeletal muscles; this was well demonstrated when only a small opening was made in the anterior abdominal wall preliminary to an electric shock. As soon as the shock was applied the intestines were forced through the opening.

When the unoperated animal was previously given atropine and then shocked, there was neither defecation, micturition nor emission, even though the abdominal muscles contracted in their usual manner. Similarly, when the peritoneal cavity was opened we observed no variation in peristalsis, in the seminal vesicles or in the bladder.

From these observations in the normal animal, the animal with the peritoneal cavity opened and the animal who had previously received atropine, it would appear that the response of the abdominal viscera was due to the combined effect of contracting skeletal muscle and contraction of the individual viscera themselves.

The Response of the Respiratory Center to Shock

The onset of the shock was accompanied by a deep inspiration, with a powerful contraction of the intercostal muscles and diaphragm. This state was maintained throughout the entire period of shock and respiratory movements did not return for several seconds afterwards when they were shallow, irregular and rather difficult to follow.

Physiologic Changes in the Neuromuscular System

Probably these were the most interesting of all the varied effects which we observed. We have touched briefly on the rather unusual nervous manifestations, namely, the excitability, nervousness and stupor, and have suggested that the responses seen in the other systems are in reality simply indirect manifestations of an altered nervous system. Now in addition to these there were three outstanding changes which belong specifically to the neuromuscular system alone, namely, weakness, paralysis of the posterior extremities and diminished sensation.

The weakness was usually not seen until after the third or fourth daily shock. The animal became less active, did not move about as quickly, appeared wobbly after a shock, and had difficulty in holding himself up.

Paralysis of both posterior extremities occurred in more than half of the test animals and usually in those who seemed rather feeble. In some cases it came on immediately after the shock; in one it appeared at once, lasted a half hour and disappeared, but again reappeared in twelve hours. Others did not become paralyzed till twenty-four to forty-eight hours had elapsed since their last shock. The paralyzed animals were watched and killed at varying intervals. One that lived fourteen days after the onset of paralysis began to regain some strength and motion in the paralyzed legs. When paralyzed the animals drag their hind feet about and appear to have no control of the bladder or anal sphincters.

If the paralyzed legs were lightly stimulated by touch, pressure, pain or heat there was no visible response. A strong electrical stimulation elicited a contraction of the muscles of the body but the normal reflex arc of the paralyzed legs had been interrupted because the leg muscles showed no response.

The left leg was always used in applying the lower electrode, and even after the first shock the animal would move this less than the other, sometimes dragging it along as though paralyzed. This we attributed to a protective mechanism to relieve the muscular pain.

We shall not attempt at this point to consider the possible pathologic conditions that might account for these neurologic findings as these will be discussed after the gross and microscopic lesions have been described. We have merely enumerated our observations to show the important rôle the nervous system plays in electrocution.

Summary of Physiologic Observations

These manifestations in normal animals, animals that had received atropine, and others with the heart and abdominal and pelvic viscera exposed, are simply expressions of the central nervous system including the craniosacral and sympathetic divisions, and may largely be interpreted on the basis of pathologic histology.

Now it is generally assumed that there are no constant demonstrable histologic lesions in the central nervous system of animals following a single fatal shock in which death is instantaneous. The microscopic study of material from cases of sudden death offers many other examples in which one finds no demonstrable lesions. The classic example is the case of chloroform poisoning; the animal that is killed with a single large dose of chloroform shows no gross or microscopic lesions in the liver, but when an animal receives one or more sublethal doses and is allowed to live twenty-four hours or longer, the degeneration, necrosis and hemorrhage in the centers of the liver lobules are startling.

This principle of giving sublethal amounts of electricity, grading the amounts and allowing the animal to live a sufficient time to permit histologic changes to appear was the method that was followed in this short series of experiments.

Pathologic Report

Complete autopsies were performed on all animals immediately after death. The only positive and constant gross finding in the animals that were given a single fatal shock was marked congestion of the lungs, liver, kidneys, spleen and heart. In the guinea pigs this finding was absent because the animals were bled at the time of death to insure better fixation of the tissues. Hemorrhages were frequently seen in the psoas, iliacus and gluteal muscles, and were usually bilateral. The meninges were injected, and two of the animals had petechial hemorrhages in meninges of both brain and cord. The spinal cord of a normal guinea pig that is autopsied immediately after death is pale, firm and homogeneous throughout. In almost every test animal there were portions of the cord that were distinctly swollen, soft and almost diffluent.

The gross findings in one animal must be described separately because this pig had been given only two shocks. Paralysis suddenly

appeared after the second shock and the animal died in eighteen hours. On examination, hemorrhages were found in the psoas and iliacus muscles, along the anterior surface of the vertebral column, in the erector spinal group of muscles, subdurally along the entire length of the cord, within the cord and also in the brain in the region of the right basal ganglia. This animal, which was the smallest animal we used in the tests, had a definite fracture of the second thoracic vertebra. This gives one an idea of the power of contracting skeletal muscle.

Sections were taken from most of the organs, namely, the heart, lungs, spleen, liver, pancreas, kidney, adrenal, testis, bladder, intestine and skeletal muscle; and of the nervous system from the eye, the cerebrum, cerebellum, pons, medulla, different levels of the cord, pituitary, posterior root ganglia and peripheral nerves.

The tissue, apart from the nervous system, was fixed only in Zenker's solution, neutral formalin and alcohol-formalin and was embedded in celloidin and paraffin. In addition to using the above-named fixatives, sections of the nervous system were also placed in Cajal's formalin-ammonium-bromide solution, Mueller's fluid, and a saturated solution of corrosive sublimate.

In staining the tissues other than those from the nervous system we used hematoxylin and eosin, eosin and methylene blue, Mallory's phosphotungstic acid hematoxylin, Mallory's aniline blue collagen stain, Sudan IV, and osmic acid with and without preliminary treatment with a weak solution of potassium bichromate. For the nervous system we employed hematoxylin and eosin, eosin and methylene blue, Mallory's phosphotungstic acid hematoxylin, osmic acid, Sudan IV, Marchi's method for degenerating myelin, Cajal's gold chloride sublimate method for neuroglia astrocytes and Penfield's second modification of the del Río-Hortega silver carbonate method for oligodendroglia.

Microscopic Report

The pathological histology in the tissues other than the nervous system is disappointing. There is congestion of the heart, spleen, kidneys and adrenals, congestion at the periphery of the lobules of the liver and congestion and petechial hemorrhages in the lungs. The lesions in the skeletal muscles that grossly showed hemorrhages and pigmentation are slight and vary with age. Scattered muscle

fibers are ruptured, the free ends are separated and for a short distance they are contracted, wrinkled, swollen, and homogeneous. Surrounding these lesions extravasated red blood cells lie free in the reticulum. After twenty-four hours polymorphonuclear and endothelial leucocytes are seen within the degenerating and necrotic portions of the muscle fibers, lying free in the stroma, and also in the perivascular tissue. In the later stages after removal of the necrotic portion of muscle fibers, regeneration of muscle occurs, showing the typical multinucleate syncytium resulting from the multiplication of muscle nuclei by direct division and the formation of new cytoplasm. Some areas show degeneration and regeneration occurring simultaneously.

No constant lesion is present in the heart. The amount of fat in the myocardium of both the "test" and "control" pigs is extremely slight and shows little variation. With Sudan IV an occasional muscle fiber is shown to contain minute fat droplets, but this finding is as common in the "control" as in the "test" animals.

The petechial hemorrhages and the capillary injection of the pia arachnoid are verified microscopically and also the hemorrhage extending along the posterior column of the cord of the animal whose vertebral column was broken.

The histologic study of the central nervous system of the animals which received a single fatal shock reveals no constant changes other than capillary injection and occasional petechial hemorrhages.

In the guinea pigs we find definite lesions that vary somewhat in the different animals, depending largely on the severity of the shocks, the number of shocks and the duration of time that elapsed before death. The lesions are best studied in the cord and peripheral nerves including the medullated nerves to the diaphragm and heart, but are also seen within the medulla, in the tracts leading from the pons to the cerebellum and also in the lower portion of the internal capsule. It is the white matter of the cord that is most seriously affected, and in the majority of animals the lesion is greatest in the posterior columns. In some it is more generally distributed throughout all the tracts, in others it seems to affect the fibers lying nearest to the gray matter, while in still others the lesion is distinctly peripheral. In the nerves only scattered fibers are affected.

The earliest change consists in a swelling of both the axis cylinders and myelin. The axis cylinder is enlarged to several times its normal

diameter. In some areas it is disintegrating and in others it has disappeared. The myelin distends its sheath and in the ordinary hematoxylin and eosin preparation has an irregular bluish mucoid appearance. As this swelling progresses the myelin gradually becomes paler till finally it disappears leaving vacuole-like spaces among the fibers.

The picture within the cord differs considerably depending on whether it is viewed from a cross-section or a longitudinal section. In the former the gray matter may show little or no change, whereas the surrounding fiber tracts which comprise the white matter may be riddled with different sized vacuoles. The axis cylinders are not present in some of the larger vacuoles, while in others they are large, irregular and eccentric; even in the comparatively normal areas they are much less uniform in size and contour than one normally finds them. In the longitudinal section the cord has the most bizarre appearance. Axones may be followed only for short distances; they are irregularly swollen and tortuous and appear to taper off at the ends as though disrupted. Myelin, in the later stages, has disappeared leaving irregular vacuoles and long spaces around and among the swollen axones.

In the more advanced lesion where the process has progressed farther, there are foci of marked myelin degeneration heavily infiltrated with phagocytic endothelial leucocytes, scattered polymorphonuclear leucocytes and a few extravasated red blood cells.

The nerves show similar though less marked lesions. Individual fibers are swollen and vacuolated with distention of the neurolemma, while the epineurium is infiltrated with numerous endothelial leucocytes.

The nerve cells from various portions of the normal guinea pig's brain and cord are distinctly different. Moreover, in studying any group of nerve cells whether in the cortex, in the medulla or in the cord, it is found that they differ in size, shape, cytoplasmic staining, in the number of their processes and also in the number, size and position of the Nissl bodies. The nuclei and nucleoli also vary in size, contour, position and staining. Because of these variations it is difficult to diagnose the early degenerative changes in the cells. However, in a few of the areas of degeneration of the cord that extend into the gray matter there is actual necrosis of the nerve cells.

So far we have described the lesion only as it appears when we

used the hematoxylin and eosin, eosin and methylene blue, and phosphotungstic acid stains. When the early lesions are stained with Sudan IV or osmic acid, endothelial leucocytes are seen distended with material resembling myelin, whereas the vacuoles in the cord remain unstained.

In both the cord and nerves of animals which lived one to two weeks after the onset of paralysis, the Marchi method for degenerating myelin produces rather remarkable pictures. There is a much more widespread degeneration of myelin than was expected from an examination of the eosin-methylene blue sections. In cross-section of the cord the degenerated myelin appears throughout the tracts in varyingly sized irregular rings, and also as small and large globules, some of which are in endothelial leucocytes. In a longitudinal section of the nerves, the even uniform distribution of the myelin is replaced by droplets lying in the neurokeratin framework.

Sections of the cord and peripheral nerves were impregnated to bring out both the oligodendroglia and microglia. There is little or no change in the oligodendroglia except in an area of degeneration where they are swollen and rather indistinct. As many as eight to ten perineuronal oligodendroglia are frequently seen surrounding an anterior horn cell in a control cord, and we never found a greater number than this in the test animals. The number of microglia is increased in the white matter, and in areas of myelin dissolution all transitional forms are found between these small cells with delicate branching projections and the large, round, phagocytic, compound granular corpuscles.

The neuroglia astrocytes stain well in both the control and test animals, and though there seems to be an increase of these elements in the posterior columns in the animals with older lesions, yet the number is so variable in different animals and in different levels of the cord that it is doubtful whether there is a beginning true gliosis as early as fourteen days.

In summing up the histologic changes we find that they are confined almost entirely to the nervous system and that they involve primarily the nerve cells, the axones and their myelin sheaths.

Discussion

The question of first importance in electrocution is "what causes death?" Now in speaking of electric currents it is usual to call those over 1,000 volts high tension and those below this, low tension currents, while the importance of either type of current in causing fatal shocks is readily shown in the number of accidental deaths occurring annually. Furthermore, individuals vary greatly in their response to electricity and different portions of the body show wide variation in their electrical resistance. The dry calloused palm of the hand may have a resistance of 2,000,000 ohms, while that of the mucous membrane of the same person may be only 100 ohms.

In our experiments we used very low voltage. When the current was applied the heart ceased beating; this, however, did not occur when a sufficient dose of atropine had been given previously. This suggests that the stopping of the heart results from unusual vagus stimulation. It is stated clinically that in death resulting from shocks of low tension the heart ceases first and respiration continues, while in high tension currents paralysis of the respiratory center is said to precede cessation of the heart beats. Death may be produced readily in guinea pigs using a low tension current, provided the exposure is long enough; with short exposure, however, the heart ceases fibrillation and the normal rhythm returns. In larger animals and man, fibrillation of the ventricle is almost always fatal and so death ensues.

It does not appear to be the heart muscle that is affected by the electric current but rather the entire nervous system. It seems reasonable to suppose that the same factors would apply for either currents of high or low tension and hence the production of apparent cardiac death simply depends on whether or not the heart has been thrown into fibrillation.

There are three possible causes which must be considered in the production of lesions within the nervous system, namely, the electric current, mechanical force and ischemia. We have seen from our experiments the relative importance of these and conclude that the electric current itself is probably the most important single factor.

Death in electric shock may occur at the time of the shock, several minutes later, or days later, or may result from complications resulting from local injury. Occasionally a patient dies suddenly hours

or days after an electric shock. This type of death was seen in the experimental animals in whom it was found that while there were no demonstrable lesions in the myocardium, there were definite lesions in the cord, peripheral nerves and nerves to the heart. Hence, we feel that though a patient may appear in normal health after a shock reasonable precautions should be taken for days and weeks to avoid unnecessary stress and strain.

Summary

1. The literature dealing with the pathologic changes in the nervous system of electrocuted animals is reviewed.

2. The observations in normal animals, animals with exposed viscera and animals under atropine which were receiving electric shock are described in detail.

3. The value of repeated sublethal shocks in obtaining histologic lesions is stressed.

4. The rôle of the nervous system in producing changes in other systems is discussed.

5. The gross and microscopic lesions are described in which it is shown that the lesions are confined almost entirely to the nervous system and skeletal muscle.

6. The discussion deals with physical properties of electrical currents, the resistance of the body to currents, the different types of death from electric shock and emphasis is laid on the importance of prolonged rest in cases of recovery.

Note: I am indebted to Dr. F. B. Mallory for his many suggestions and helpful criticism, and also for the photomicrographs; and to Miss Marion E. Lamb for technical assistance.

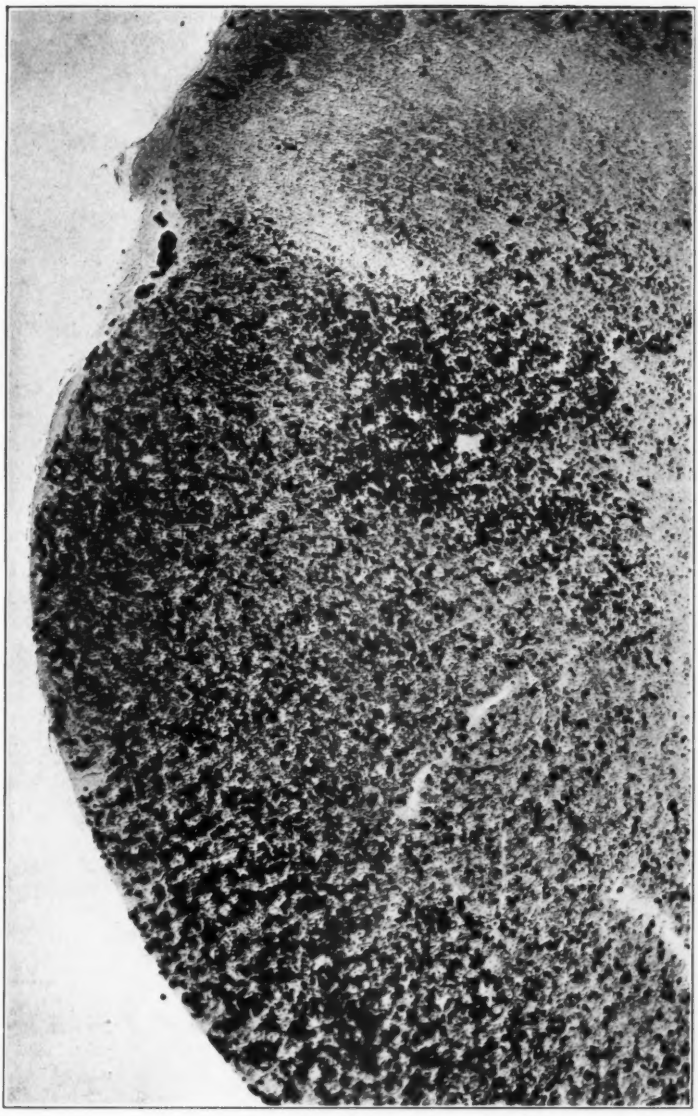
References

1. Jaffé, R. H. *Arch. Path. & Lab. Med.*, 1928, v, 837.
2. Spitzka, E. A. *Proc. Am. Phil. Soc.*, 1908, xlvii, 39.
3. Werner, A. H. *New York M. J.*, 1923, cxviii, 498.
4. Corradi, T. *Atti d. r. Accad. med.-chir. di Napoli*, 1898, lii, 1.
5. Mott, F. W., and Schuster, E. *Proc. Roy. Soc. Med.*, 1909–10, iii, 140.
6. Capogrossi, A. *Arch. internat. de méd. lég.*, 1910, i, 236.
7. Kawamura, I. *Ztschr. f. d. ges. exper. Med.*, 1921, xii, 168.
8. Gubler, E. *Deutsche Ztschr. f. d. ges. gerichtl. Med.*, 1926, viii, 406.
9. Jellinek, S. *Compt. rend. Acad. d. sc.*, 1925, clxxxi, 945.
10. Bolognesi, G. *Arch. ital. de biol.*, 1915, lxiv, 149.
11. Pietrusky, F. *Deutsche Ztschr. f. d. ges. gerichtl. Med.*, 1925–26, vi, 535.

DESCRIPTION OF PLATES

PLATE 68

FIG. 1. Section of cord removed fourteen days after the onset of paralysis and stained by Marchi's method for myelin degeneration. There is marked myelin degeneration in the lateral tracts, while just beneath and partly involving the posterior horn is an area of "softening" in which the degenerated myelin droplets have coalesced; many of them are in phagocytic endothelial leucocytes. × 100.



1

PLATE 69

FIG. 2. Photomicrograph of a longitudinal section of the cord showing in the posterior columns the swollen, tortuous axis cylinders. The clear areas represent swollen myelin or areas from which myelin has entirely disappeared. There is a moderate infiltration of endothelial leucocytes. (Hematoxylin and eosin.) × 300.

FIG. 3. Photomicrograph showing an area of "softening" in the posterior column of the cord. The normal morphology and arrangement of the elements are destroyed. Axis cylinders and myelin have disappeared; the latter is in part phagocyted by endothelial leucocytes. (Hematoxylin and eosin.) × 250.

2

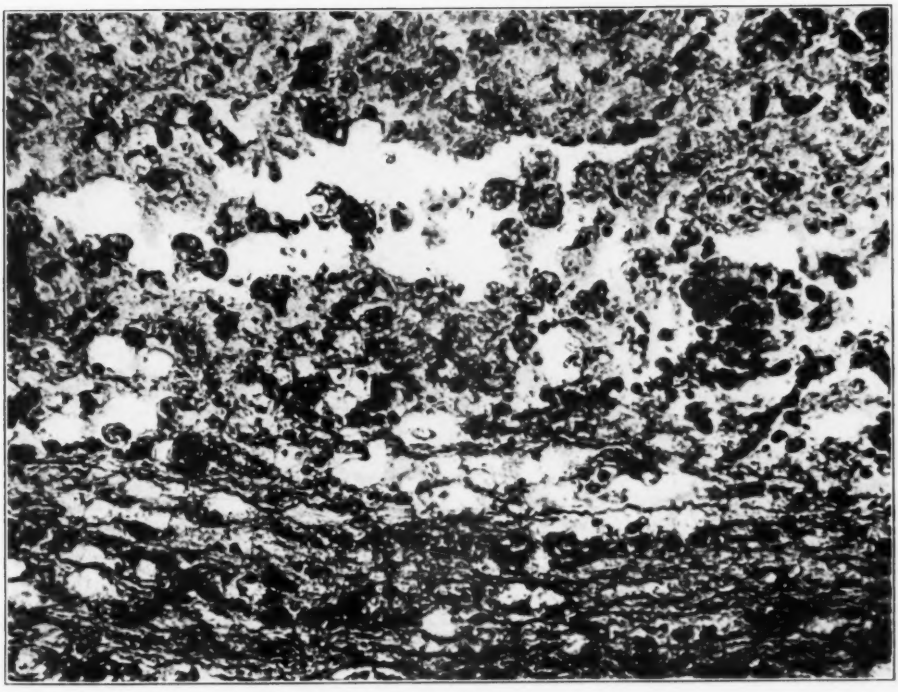


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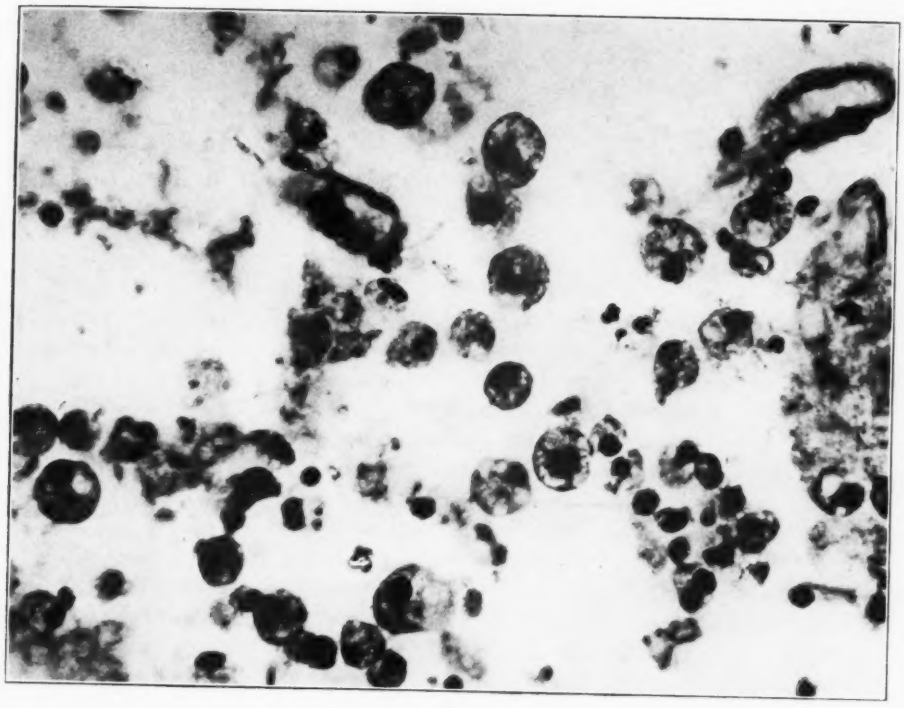
Plate 70

Fig. 4. A higher power photomicrograph from an area of degeneration in the cord, showing endothelial leucocytes many of which are distended with myelin. × 500.

Fig. 5. Longitudinal section of a peripheral nerve traversing bundles of striated muscle. Degenerated myelin droplets of various sizes are held in the neurokeratin framework of the nerve fibers. Marchi stain for degenerated myelin. × 125.



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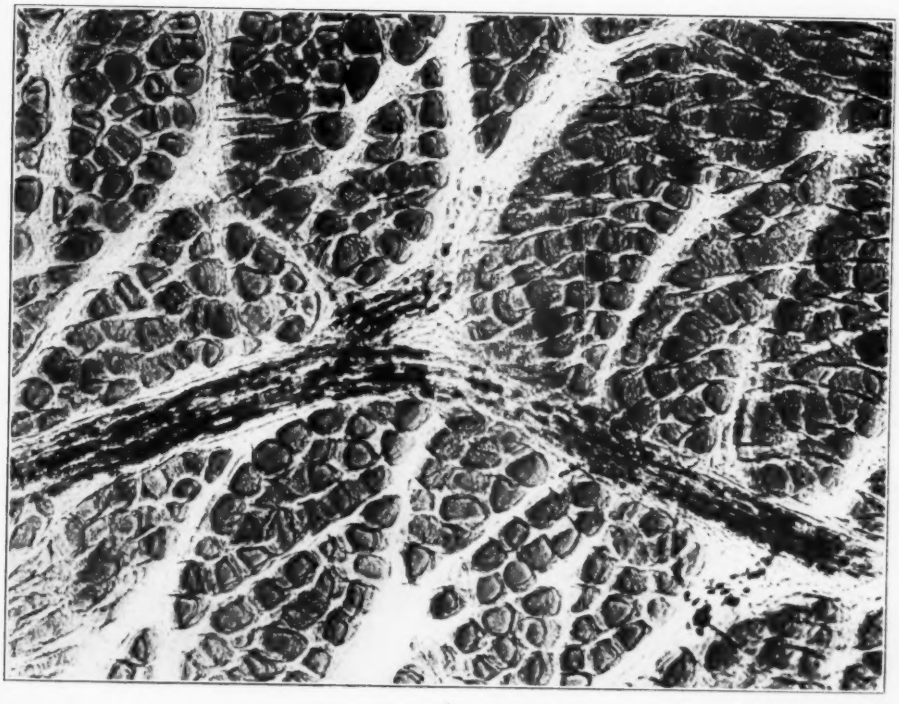

5

MacMahon Electric Shock

A CASE OF MENINGITIS DUE TO ENDOMYCES CAPSULATUS (NEW SPECIES) *

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The purpose of this paper is to report the clinical record and autopsy findings of a fatal case of systemic *Endomyces capsulatus* infection. The organism was isolated from the tissues and cultured. This paper includes a study and description of the morphology, cultural characteristics and animal pathogenicity of the organism.

Fungi may attack any organ or system of the body, the integumentary system being most frequently and the nervous system most rarely affected. Diseases of fungus origin are called mycoses. These may be classified into two groups — internal mycoses and external mycoses or dermatomycoses.

Castellani [1] has stated that in cases of general infections due to fungi like Saccharomyces, Monilia and Endomyces, abscesses containing such fungi may be found in the brain and spinal cord. In this country cases of cerebrospinal-meningitis have been recorded due to *Cryptococcus histolyticus* (synonym *Torula histolyticus*), Stoddard and Cutler,[2] 1916. It has been shown that Torula has a special predilection for the central nervous system and lungs.

Summaries of our knowledge concerning *Torula histolytica* infections in man have been made from time to time since Stoddard and Cutler's monograph was published in 1916. The more recent reports are by Shapiro and Neal [3] in 1925, and Rappaport and Kaplan [4] in 1926. Shapiro and Neal mentioned thirteen cases then reported. They added another case and included a personal communication from Sheppe. This made the total reported cases fifteen up to that time, of which thirteen were infections of the central nervous system and meninges, one of the lung, and one of the muscles of the back. Rappaport and Kaplan reported a systemic torulosis and McKendree and Cornwall [5] in the same year reported an infection of the brain

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and meninges. An account by Hansmann [6] in 1924 of a case of meningo-encephalitis caused by *Torula histolytica* was not mentioned by these writers. About this time Lynch and Rose [7] reported an additional case of meningo-encephalitis due to *Torula histolytica*. Hall, Hirsch and Mock [8] in 1928 added a case of meningo-encephalitis. Wortis and Wightman [9] in 1928 reported another case of *Torula histolytica* meningitis. Hence we have collected a total of eighteen cases in which *Torula histolytica* was the etiologic factor in meningitis.

A case of sporotrichosis meningitis was reported by Hyslop, Neal, Kraus, and Hillman [10] in 1926.

Vialard, Lancelin, and Darleguy [11] in 1925 reported a case of meningo-encephalitis due to Trichomyces in a case of pulmonary tuberculosis.

In 1917 Swift and Bull [12] reported a case of primary blastomycotic meningitis. Since then in 1922 Williams [13] has recorded a case of meningitis which followed a case of pulmonary blastomycosis. Badham [14] has mentioned a case which appeared in Sydney in 1922. Watanabi [15] reviewed a case in which blastomycotic invasion of the tonsils was reported.

Report of Case

Clinical History: J. M. (Hospital No. 568,911), a white, married male, 48 years of age, who was a furrier by trade, entered the Boston City Hospital, August 30, 1928, complaining of continuous frontal and temporal headaches during the past three and a half months.

Family history was negative, and the past history was without interest.

His present illness began five months before entry when he noticed a hard tender slightly movable mass about the size of a hen's egg on the medial surface of the lower third of the left thigh. It gradually subsided during the next four weeks, at the end of which time a similar mass appeared beneath the anterior surface of the neck. A short time after the first mass subsided the patient began to have continuous and almost unbearable frontal and temporal headaches. Two weeks before entry he developed defects in memory, would lose himself on familiar streets, could not remember places and lost track of time. He lost interest in everything and seemed not to understand questions or conversation. He gradually became worse and was brought to the hospital.

Physical Examination: A well developed and emaciated middle-aged white man who was dull mentally and slightly confused. A tender cystic mass about the size of a hen's egg was situated at the level of the thyroid isthmus between the trachea and the anterior border of the left sternocleidomastoid muscle. The chest was symmetrical. Resonance over both lungs was impaired and over the right apex were heard both fine and medium crepitant rales. A small tender cystic mass about the size of a walnut was felt on the medial surface of the lower

third of the left thigh. Temperature, 98.6° F; pulse, 84; respiration, 20; blood pressure, 130 systolic and 80 diastolic.

Neurologic Examination: The pupils were contracted and reacted slowly to light and accommodation. The fundi showed perivascular streaking, venous engorgement and slight pallor of both discs. Bilateral diminution of hearing. The deep reflexes were exaggerated more markedly on the right. The cremasteric and Babinski reflexes were absent. During the examination the patient was very emotional, having fits of crying and laughing.

Laboratory Findings: The urine and blood examinations were negative except for a 5 per cent eosinophilia. The blood Kahn was negative. The spinal fluid showed an initial pressure of 210 mm., a slow fall after abdominal and jugular compression, leucocytes 20, of which 15 were polymorphonuclear and 5 lymphocytes, Wassermann positive and gold sol. 0000022220. The spinal fluid was cultured on blood agar, chocolate agar and Sabouraud's medium, and showed no growth at the end of fourteen days. A smear of this spinal fluid was negative. The second spinal fluid was similar except that the pressure and dynamics were normal and the gold sol. 0001242110. Culture of the spinal fluid on blood agar, chocolate agar and Sabouraud's medium, showed no growth at the end of fourteen days.

September 11, 1928: The cyst in the thyroid region was aspirated, and 30 cc. of thick creamy tenaceous yellow pus was obtained. Smears of this pus contained numerous polymorphonuclear leucocytes and countless numbers of round granular cells with vacuoles and refractile bodies in their cytoplasm. These cells were encapsulated and were one fourth the size of a red blood cell. Numerous budding forms were present. The organism was cultured and identified as *Endomyces capsulatus* (new species). Organisms, which were identical morphologically and culturally with the organisms cultured from the pus from the neck, were found in the pus aspirated from the nodule on the left thigh.

September 16: X-ray report: extensive bilateral pulmonary tuberculosis; skull plates were negative.

After a short stay the patient left the hospital against advice when a biopsy was suggested. His condition was unchanged.

Clinical Diagnoses: Mycotic encephalitis(?); mycotic abscesses of the neck and left thigh; bilateral pulmonary tuberculosis.

October 3, 1928: The patient was readmitted.

Interval History: (Hospital No. 571,024). Since his discharge the patient had become completely disoriented. A week before entry he became incontinent and lethargic.

Physical Examination: The patient was comatose and had mild choking of the discs. He had a stiff neck and bilateral Kernigs. Babinski's sign was positive on the left and questionable on the right. Temperature, 98.6° F; pulse, 80; respiration, 20.

Laboratory Findings: The urine and blood examinations were negative except for a 4 per cent eosinophilia in one smear. Spinal fluid showed an initial pressure of 110 mm., the dynamics were normal, total protein 171 mg., leucocytes 51, of which 62 per cent were lymphocytes, gold sol. 2233443100, chlorides 568 mg. and sugar 36 mg. Culture of the spinal fluid on blood agar, chocolate agar and Sabouraud's medium showed no growth at the end of fourteen days. The second spinal fluid showed chlorides 579 mg., sugar 39 mg., total protein 190 mg., and leucocytes 68. Culture, guinea pig, and mouse inoculations were negative. These animals were killed and autopsied two months after the inoculation.

The patient's condition became worse, his temperature, pulse and respirations rose rapidly, and he died October 31, 1928.

Clinical Diagnoses: Mycotic meningo-encephalitis(?); bilateral pulmonary tuberculosis.

An autopsy (A–28–350) was performed eleven hours postmortem. The tissues were immediately placed in Zenker's fixative and later embedded in paraffin and stained with eosin-methylene blue, phosphotungstic acid hematoxylin, Mallory's aniline blue, Gram Weigert's method and by Ziehl–Neelson's method for tubercle bacilli in paraffin sections.

Autopsy Report

The body is that of a well developed and emaciated middle-aged white man. A red scar 1.5 cm. long is present to the left of the midline on the anterior surface of the neck. A similar scar is present on the medial surface of the lower third of the left thigh. The left femoral lymph nodes are palpable.

Peritoneal Cavity: On the serosal surface of the ileum are two congested areas 2 cm. in diameter, and a third is present on the cecum near the ileocecal valve. In these congested areas are numerous firm gray nodules measuring about 0.1 cm. in diameter. The remainder of the peritoneal cavity is without interest.

Pleura and Lungs: The pleural surfaces are smooth and glistening save for the left apex where there are dense fibrous adhesions joining the visceral and parietal pleural surfaces. Numerous firm irregularly shaped and sized nodular masses are present throughout both lungs involving the upper lobes more than the lower. Fresh section of these firm masses reveals numerous closely approximated firm yellowish white nodules measuring 0.1 cm. in diameter. No cavity formation present. The bronchi and the mediastinal structures are negative.

Heart and Spleen: Without interest.

Gastro-Intestinal Tract: Stomach negative. Two ulcerative lesions in the lower ileum and a third in the cecum near the ileocecal valve are similar in appearance. In the ileum they entirely encircle the bowel and are 1.5 to 2 cm. broad. The margins of the ulcers are ragged, nodular, irregular, thickened and undermined. Their bases are covered with yellow necrotic material. The serous surface is congested, and numerous gray nodules about 0.1 cm. in diameter are present in the subserous tissues.

Liver, Pancreas, Kidneys, Adrenals, Bladder and Genital Organs: Without interest.

Organs of the Neck: The thyroid, trachea, esophagus and larynx are negative. To the left of the midline the subcutaneous tissues are flesh-colored, granular and are studded with numerous firm nodules 0.2 cm. in diameter.

Left Thigh: The subcutaneous tissues beneath the scar on the left thigh are flesh-colored, granular and are studded with numerous firm nodules measuring 0.2 cm. in diameter. The tissue involved is about 2 cm. in diameter.

Head: The scalp and calvarium are negative.

Brain: Weight 1,120 gm. The dura is tense. When opened the hemispheres bulge out and when uncovered present a flattening of the convolutions and an obliteration of the sulci. The cerebrospinal fluid in the subarachnoid space is clear. The lower surface of the cerebellum, pons, medulla and the cranial nerves arising from the brain stem are covered with numerous granular, closely approximated, firm, flesh-colored nodules. No abscesses or nodules are observed in the brain substance. The ependyma is rough and granular. The ventricles are moderately dilated.

Anatomic Diagnoses: Nodular meningitis; granular ependymitis; nodular cellulitis of the neck and left thigh; bilateral pulmonary tuberculosis; tuberculous ulcers, ileum and cecum.

Microscopic Examination

Lung: The normal architecture is obliterated by roughly concentric lesions. Endothelial leucocytes and foreign body giant cells are present in and around central zones of necrosis which are composed of shadowy remains of cells. Acid-fast bacilli are present in the necrotic centers. At the periphery of the lesions is a narrow border of lymphocytes. Conglomerate lesions similar in structure are present. The adjacent alveoli are filled with endothelial leucocytes.

Heart and Spleen: Without interest.

Intestine: An ulcer with an undermined edge extends down to the muscularis. In the base of the ulcer and extending out beneath the undermined edge are lesions similar to those seen in the lung. Acid-fast bacilli are present in the necrotic centers of the lesions.

Liver: Small discrete lesions similar to those seen in the lung and intestine are present.

Pancreas, Kidney, Adrenal, Prostate, Thyroid and Pituitary: Without interest.

Subcutaneous Nodule, Left Thigh: In the subcutaneous tissues there are roughly concentric and conglomerate lesions composed of endothelial leucocytes and one or more foreign body giant cells and surrounded by a narrow border of lymphocytes. A moderate amount of connective tissue surrounds the lesions. In the larger affected areas are foci of necrosis in which are found many polymorphonuclear leucocytes, fewer vacuolated endothelial leucocytes, and an occasional foreign body giant cell.

In the cytoplasm of the endothelial leucocytes and foreign body giant cells are round yeast-like organisms which are surrounded by a clear non-staining capsule. These organisms vary from 0.7 to 2.8 microns and average 1.6 microns in diameter. Within the organisms are round and oval basic-staining bodies resembling nuclei. In the small organisms only one is present, while in the larger ones two and even three have been observed. In sections stained by Mallory's aniline blue the capsule of the organisms stains blue. The organisms are gram positive.

Within the tissues this organism reproduces by budding. By studying the organism in the foreign body giant cells and endothelial leucocytes, all stages of budding have been observed. An early stage is characterized by a slight protoplasmic bulge at the periphery of the organism accompanied by a similar bulge of the capsule. In another cell may be seen a well developed bud which is still joined to the parent cell. A later stage is observed in which a capsular septum separates the daughter from the parent cell. In the last stage the separation is complete, each cell having its own capsule.

Medulla: On the pial surface of the medulla and surrounding the cranial nerves there are roughly concentric and conglomerate lesions composed of endothelial leucocytes and one or more foreign body giant cells and surrounded by a narrow border of lymphocytes. In the larger lesions are areas of necrosis in which are found many polymorphonuclear leucocytes, fewer vacuolated endothelial leucocytes and an occasional foreign body giant cell. In the cytoplasm of the foreign body giant cells and endothelial leucocytes are round yeast-like organisms, surrounded by a clear non-staining capsule. These organisms are similar to those seen in the tissues of the thigh.

Microscopic Diagnoses: Mycotic meningitis; mycotic cellulitis, left thigh; tuberculosis of lungs; tuberculous ulcer, ileum; miliary tuberculosis, liver.

Bacteriologic Study

A nodule removed from the pial surface of the medulla was washed in several changes of sterile saline, ground in a mortar, suspended in saline and cultured on Sabouraud's medium. Colonies of Endomyces were observed on the seventh day. Two mice were inoculated with this suspension. The first mouse died on the sixth day, and the second on the seventh. Gross examination was negative except for multiple abscesses in the kidneys. Microscopic examination was negative except for the kidneys. Large areas in which the normal architecture was destroyed were infiltrated by polymorphonuclear and a few endothelial leucocytes. Endomyces within endothelial leucocytes were present in the abscess and in the adjacent tubules.

B. Coli was cultured from the heart's blood and spleen.

Morphology

Methods: Fleming's weak killing and fixing solution was added directly to the agar tube containing the fungus. After remaining in this solution for about twenty-four hours, the material was washed in running water from twelve to twenty-four hours. After washing, the material was removed from the culture tube, a thin slice of agar containing the fungus on one surface was cut from the agar plug, transferred to commercial hydrogen peroxide (3 per cent) and bleached. This material was then stained either with Delafield's hematoxylin or with Heidenhain's iron alum hematoxylin and counterstained with an aqueous solution of eosin. When the material was satisfactorily stained it was transferred to a 10 per cent aqueous solution of glycerol which was allowed to concentrate by evaporation of the water until an equilibrium was reached. The material was then mounted on slides, gently teased apart as much as possible with fine needles and a cover glass added.

Endomyces Capsulatus, Rewbridge, Dodge and Ayers: New Species

The mycelium in lesions consists exclusively of budding cells; see Figs. 22, 23 and 24. In culture, when first isolated, and in acid media, the mycelium consists of coarse thin-walled hyphae, 3.5 to 4 microns in diameter, composed of nearly isodiametric cells (Fig. 3). Hyphae, often somewhat contorted, suggest the mycelium of Madurella. On neutral media with protein as the principal source of nitrogen the hyphae are slender, branched, 1.4 to 1.5 microns in diameter with long, mostly uninucleate cells.

Budding cells about 3.5 by 5 microns are abundant on acid media, but absent on neutral media (Fig. 3).

Hypnospores (chlamydospores) terminal on short branches or in chains, develop from mycelium resembling the raquet mycelium of Trichophyton and related genera. The cells of this mycelium are 6.5 to 7 microns in diameter at the swollen end and 2.6 to 3 microns in diameter at the other end (Figs. 15 and 19). In cultures on cornmeal agar, large spherical cells, 11.3 microns in diameter, develop on the ends of short branches. They have very thick walls but their contents seem to be degenerating (Fig. 1).

Conidia are sessile, pyriform, 5.5 by 6.5 microns, developing irregularly along the mycelium but usually near a septum, more abundant on media with protein nitrogen and a neutral reaction (Figs. 14 to 16).

Endospores (*endoconidies* of Vuillemin[16], *globules internes* of Salvat and Fontoynont[17]) are ellipsoidal, 2.6 by 3.2 microns in the hyphae on Sabouraud's dextrose agar (Fig. 12). These spores are formed by the rounding up of the protoplasts into deeply staining, ellipsoidal masses, but no spore wall was observed and their significance is still uncertain. Their appearance suggests that of the hormogonia of the Myxophyceae.

Sexual reproduction when present is heterogamous. A cell of a filament swells to about 11 microns in diameter and fuses with an apparently little differentiated cell (4.8 microns) on a hypha (3.6 microns) which may lie nearby (Figs. 6, 7, 9). Whether this is a case of amphimixis or pseudomixis or whether it is related to the curious sexuality of *Ascobolus carbonarius* reported by Dodge[18] must await a further study of the nuclear divisions preceding and during this

copulation. Since pseudomixis is common among the yeasts it might be expected here.

The details of development between copulation and the formation of the asci have not been sufficiently studied. The asci are terminal on long filaments, eight-spored, subspherical, about 7 by 9 microns. The ascospores are hyaline, spherical, smooth, 2 microns in diameter (Fig. 4). So far, asci have occurred only in cultures on Sabouraud's dextrose agar about nineteen weeks old.

Cultural Characteristics

The original isolation furnished us from this case was cultivated at room temperature (about 20° C). It was transferred to a variety of media roughly ranging from pH 4.5 to 7.2. Since our laboratory is poorly equipped for accurate determination of the concentration of hydrogen ions, in this preliminary work we have been content to accept the statement on the labels of recent batches of dehydrated media from the Digestive Ferments Company and will postpone further detailed consideration of the physiology of this fungus to a later paper.

Since a cursory examination of the original culture showed the general morphology of a Monilia or of some other imperfect yeast, we tried it on a number of acid media which are usually satisfactory for this group. It will be seen, however, that for this organism media with a lower hydrogen ion concentration and more complex nitrogenous compounds were more satisfactory. Since most of our time has been devoted to the morphology of the organism we have not yet attempted to study its growth on slightly alkaline media, but since our best growth occurred on Bacto Nutrient (beef) agar at pH 7.2, it is probable that growth will occur on more alkaline media.

In the following descriptions of colonies the media have been arranged approximately in the order of decreasing concentration of hydrogen ions.

Wort Agar (Bacto pH 4.5): No growth.

Wort Agar: (Approximately the formula published for Bactowort agar with slight increases in amounts of maltose, dextrin and [Bacto] peptone, which would probably cause a slight decrease in concentration of hydrogen ions.) Surface of the colony moist, shining, yeast-like at first, becoming dull or somewhat powdery in age.

Growth slow, reaching less than 1 cm. in diameter in six weeks, elevated, convolute to cerebriform. Mycelium very irregularly branching, septate; cells very short (Fig. 3). This culture after five months resembles most closely the freshly isolated cultures.

Malt Extract Agar (*Bacto pH about 4.7*): Growth slow, scarcely covering the surface of the tube in nineteen weeks, forming a compact thin felt in the older portion, with only a very sparse or fine powdery surface in the younger portion. Mycelium more filamentous than in wort agar with very few yeast cells.

Sabouraud's Dextrose Agar (*Bacto pH 4.74*) *and Sabouraud's Conservation Agar:* Growth more rapid, the colony reaching 2 cm. in diameter in two weeks, white, well developed at the inoculum, surrounded by a loose cottony zone with a dense cottony periphery. Mycelium filamentous, hyaline, branched, composed of clavate cells, 2 to 4.5 microns in short diameters. Hyphal tips swollen, suggestive of the raquet mycelium of Trichophyton. It should be noted that the original cultures on Sabouraud's agar were less definitely filamentous and more yeast-like. It is not clear whether that particular batch of agar was more acid or whether frequent transfers on artificial substrates have gradually adapted the fungus to a changed environment. The writers are inclined to the former alternative in view of the growth on media of higher concentrations of hydrogen ions.

Corn-meal Agar (*Bacto pH 5.9*): Growth similar to that on Sabouraud's agar but much less developed and much slower, covering less area in eight weeks than it does on Sabouraud's medium in two weeks. Mycelium very slender, cells very long. Thick-walled hypnospores abundant.

Swordfish (*Sterilized Muscle of Xiphias Gladius L., pH 6.1*): Growth very slow, colony less than 0.7 cm. in diameter in three weeks, more compact than colonies on corn-meal agar, quite elevated. Mycelium slender, cells long, producing abundant conidia, many of which are terminal on short branches, arising at right angles to the hyphae as in Acremonium.

Cooked Egg: (The whites and yolks were beaten together, coagulated, then tubed and sterilized at a pressure of 15 lb. per square inch for twenty minutes; pH unknown.) Growth slow, similar to that on swordfish but conidia much less abundant.

Beef Agar (*Bacto Nutrient pH 7.2*): Growth rapid, forming a

thick, smooth felt over the whole surface. Mycelium very slender; conidia abundant.

Animal Pathogenicity

Guinea pigs, rabbits and mice were used for study. A culture of *Endomyces capsulatus* on a Sabouraud slant incubated at 37° C for seven days was suspended in 20 cc. of saline. Suspensions made in this way were used in the following animal experiments.

Each one of four guinea pigs was inoculated intraperitoneally with 4 cc. of the above described suspension. At the end of eleven weeks they were healthy and had not lost any weight. They were then killed and autopsied. The four guinea pigs were grossly and microscopically negative. Cultures of the heart's blood on Sabouraud's medium and blood agar showed no growth at the end of seven days.

Each one of four rabbits was inoculated intravenously with 4 cc. of the above-described suspension. At the end of twelve weeks they were healthy and had not lost any weight. They were then killed and autopsied. The four rabbits were grossly and microscopically negative. Cultures of the heart's blood on blood agar and Sabouraud's medium showed no growth at the end of seven days.

Each one of four mice was inoculated intraperitoneally with 0.5 cc. of the above described suspension. These mice developed a progressive infection, became emaciated and died between the third and fourth weeks. The lesions found at autopsy were identical.

Gross Examination: Numerous firm granular nodules measuring 0.2 to 0.3 cm. in diameter were present in the omentum and on the peritoneal surface. Similar granular nodules were observed in the lungs, liver, spleen and kidneys.

Microscopic Examination: In several large areas the normal architecture of the lung is destroyed, and the cavity formed is filled with polymorphonuclear leucocytes, a few endothelial leucocytes and countless numbers of encapsulated Endomycetes. Numerous budding cells are present. Similar lesions are present in the liver, spleen, kidneys and omentum.

Cultures of the heart's blood on blood agar and Sabouraud's medium showed no growth at the end of seven days. Peritoneal nodules were cultured on Sabouraud's medium. Colonies of Endomyces were observed on the seventh day.

Summary of Animal Experiments

1. Mice when inoculated intraperitoneally with *Endomyces capsulatus* develop a progressive infection affecting the peritoneum, lungs, liver, spleen and kidneys, resulting in death at the end of three to four weeks.

2. The organism Endomyces can be cultured from the lesions.

3. The organism was not cultured from the heart's blood of mice killed by this infection.

4. Rabbits after receiving an intravenous inoculation were healthy and had not lost any weight at the end of twelve weeks. They were grossly and microscopically negative.

5. Guinea pigs after receiving an intraperitoneal inoculation were healthy and had not lost any weight at the end of eleven weeks. They were grossly and microscopically negative.

Classification

The Saccharomycetales, to which the organism here studied belongs, are divided into three families: the Dipodascaceae, a primitive family with several features which are suggestive of the higher Phycomycetes; the Endomycetaceae or filamentous yeasts; and the Saccharomycetaceae or true yeasts. Besides these there are a large number of organisms whose life cycles are incompletely known which probably belong to these groups. From a survey of the medical literature dealing with these organisms, the nomenclature appears chaotic and the application of generic names uncertain. It seems best to the writers to consider species having a variable amount of filamentous mycelium, which shows the characteristic budding associated with the yeasts, as belonging to Monilia, and the species with spherical or nearly spherical cells which may adhere in chains but which never form characteristic filamentous mycelium as Cryptococcus. While it is never safe to predict the genus of Ascomycetes to which one of the Fungi Imperfecti will belong when the whole life cycle is known, it is probable that the species of Monilia will belong to Endomyces and those of Cryptococcus to the Saccharomycetaceae.

The Endomycetaceae are usually divided into about a half-a-dozen genera of which only two, Eremascus and Endomyces are at all well known cytologically. Eremascus, found on fruit juices, lacks

the characteristic budding of Endomyces and has regular isogamous reproduction.

Endomyces which grows on a wide variety of substrates shows great variation in its reproduction. The species may be divided into two series, one of which has retained its primitive isogamy and gradually degenerated until the sexual act becomes rare and parthenogamy the rule; the other series starting with quite well defined heterogamy as in the present species has also degenerated towards parthenogamy, so that the end results of the two series are identical or at least so similar that it is difficult to differentiate them.

If we exclude species whose ascogenous stage is unknown, species which have often been included by their authors in Endomyces, very few species have been found in man or other mammals. Some of these as *E. Cruzi* De Mello and Paes, and *E. pulmonalis* Senez were described in journals to which we have not had access. The second, perhaps, belongs in Monilia. While we have not seen the original description of *E. bonaerensis* Greco,[19] the discussion by its author (1916) leads us to place it either along with *Monilia alba* (Queyrat and Laroche [20]) Sartory [21] (1922) from vaginitis (not the organism described under that name by Castellani in 1911), or with *E. Vuillemini* Landrieu, closely related to *Monilia albicans* of thrush and similar infections. During the campaign in Mesopotamia, *E. tropicalis* Castellani and Acton [22], which produced creamy patches on tonsils and uvula that were difficult to remove but left no raw bleeding surfaces and were accompanied by diffuse inflammation of the uvula, of pillars of the fauces and of the posterior pharyngeal wall, was described as having a very curious life cycle. If the description should prove to be correct, it should certainly be removed to a new family if not to a new order of fungi. It certainly has little in common with the organism under consideration.

E. crateriforme Hudelo, Sartory and Montlaur [23] produced an oval, erythematous, scaly lesion in the armpit of a young woman reminding one of dry seborrheic eczema. It was not pathogenic to guinea pigs or rabbits by subcutaneous, intraperitoneal or intravenous injection, although scarification produced non-typical evanescent lesions. It is evident that it was unable to invade the deeper tissues of the body differing markedly from *E. capsulatus*.

Only one true Endomyces, *E. Molardi* Salvat and Fontoynont [17, 24] remains for discussion and separation from our organism. The

former was reported from a lesion on the leg, originally hard nodules which softened into an abscess which was finally cured after about two months by external application of methylene blue. Inoculation in the thigh of a guinea pig produced a local abscess which healed spontaneously. Intraperitoneal injections were fatal in thirty-three to forty-four days with cachexia accompanied by nephritis. The organism was recovered from the blood.

The mycelial filaments are 2 to 6 microns in diameter, somewhat contracted at the septa, and thick-walled. The filaments may end either in a simple cell; a group of short thick-walled, nearly spherical, yeast-like cells, sometimes twice the diameter of the penultimate cell; or by a spherical cell 8 by 22 microns in diameter. The latter are evidently hypnospores and sometimes occur in groups or short chains. The asci and ascospores are produced on glycerol agar or on carrot plugs with glycerol. The asci vary from spherical to ellipsoidal with four, rarely eight spores.

On glycerol agar the growth was rapid, thick, creamy or ivory white, elevated, with small deeper yellow craters in the middle. On Sabouraud glucose agar growth was less abundant, consisting of plateaux slightly raised in the center, creamy white, glistening, then opaque, with fine radiations from the central cone about the fifteenth day. A comparison of *E. Molardi* with our organism shows differences in most points of morphology, culture and pathogenicity, although it is certainly congeneric.

Summary

1. The clinical record and autopsy findings of a case of meningitis due to *Endomyces capsulatus*, n. sp., are reported.

2. The organism was not found in the spinal fluid.

3. The morphologic and cultural characteristics of *Endomyces capsulatus*, n. sp., are described.

4. The organism is pathogenic for mice but not for rabbits or guinea pigs.

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REFERENCES

1. Castellani, A. Fungi and fungous diseases. *Arch. Dermat. & Syph.*, 1928, xvii, 91–92.
2. Stoddard, J. L., and Cutler, E. C. *Monograph of the Rockefeller Institute for Medical Research*, vi, 1916.
3. Shapiro, L. L., and Neal, J. B. Torula meningitis. *Arch. Neurol. & Psychiat.*, 1925, xiii, 174.
4. Rappaport, B. Z., and Kaplan, Bertha. Generalized Torula mycosis. *Arch. Path. & Lab. Med.*, 1926, i, 720.
5. McKendree, C. A., and Cornwall, L. H. Meningo-encephalitis due to Torula. *Arch. Neurol. & Psychiat.*, 1926, xvi, 167.
6. Hansmann, G. H. Torula infection in man. Report of a case. *Boston M. & S. J.*, 1924, cxc, 917.
7. Lynch, F. B., and Rose, E. Torula meningitis; report of an additional case. *Ann. Clin. Med.*, 1925–26, iv, 755.
8. Hall, G. W., Hirsch, E. F., and Mock, H. E. Torula histolytica meningo-encephalitis. *Arch. Neurol. & Psychiat.*, 1928, xix, 689.
9. Wortis, S. B., and Wightman, H. B. Case report of Torula meningitis. *Bull. N. Y. Acad. Med.*, 1928, iv, 531.
10. Hyslop, G. H., Neal, J. B., Kraus, W. M., and Hillman, O. A case of sporotrichosis meningitis. *Am. J. M. Sc.*, 1926, clxxii, 726.
11. Vialard, Lancelin, and Darleguy. Sur un cas de méningo-encéphalite oosporique survenue chez un tuberculeux pulmonaire cavitaire. *Bull. et mém. Soc. Méd. d'hôp. de Paris*, 1924, xlviii, 1284.
12. Swift, H., and Bull, L. B. Notes on a case of systemic blastomycosis — blastomycotic cerebro-spinal meningitis. *M. J. Australia*, 1917, ii, 265.
13. Williams, J. R. A case of systemic blastomycosis. *M. J. Australia*, 1922, ii, 185.
14. Badham, C. Preliminary report on the causal organism of blastomycotic meningitis in Australia. *M. J. Australia*, 1922, ii, 385.
15. Watanabi. A case of blastomycotic invasion of the tonsils. *Medical Annual*, 1922.
16. Vuillemin, Paul. Les formes du champignon du muguet. *Rev. Myc.*, 1899, xxi, 43.
17. Salvat, P., and Fontoynont, M. Contribution à l'étude des mycoses malgaches. Abcès sous-dermiques dus à l'Endomyces molardi (n. s.). *Bull. Soc. de path. Exot.*, 1922, xv, 311.
18. Dodge, Bernard O. Methods of culture and morphology of the archicarp in certain species of the Ascobolaceae. *Bull. Torrey Bot. Club*, 1912, xxxix, 139.
19. Greco, Nicolas V. Origine des Tumeurs (Etiologie du Cancer, etc.) et Observations des Mycoses (Blastomycoses, etc.) argentines, Buenos Ayres, 1916.
20. Queyrat, Louis, and Laroche, Guy. Sur une mycose vaginale (Parendomyces albus). Étude microbiologique, experimentale et therapeutique. *Bull. et mém. Soc. méd. d'hôp. de Paris*, 1909, Series 3, xxviii, 111.

21. Sartory, A. Champignons Parasites de l'Homme et des Animaux, 1922, x, 707.

22. Acton, H. W. Mycotic affections of the throat due to Endomyces tropicalis. *Indian J. M. Research*, 1919, vi, 591.

23. Hudelo, L., Sartory, A., and Montlaur, H. Epidémiomycose eczématoïde due à un parasite du genre Endomyces. *Compt. rend. Acad. d. sc.*, 1920, clxx, 1086.

24. Fontoynont, M. Sur quelques lésions mycosiques observées à Madagascar. *Bull. et mém. Soc. de Chir. de Paris*, 1922, xlviii, 439.

DESCRIPTION OF PLATES

Plate 71

The interpretation of some of our figures must await further cytological study: Fig. 1 from corn-meal agar, Fig. 3 from wort agar, Figs. 14 and 16 from malt extract agar, the others from Sabouraud's dextrose agar.

Fig. 1. Large, thick-walled cells produced on corn-meal agar, function not yet known. × 1100.

Fig. 2. Hypha on Sabouraud's dextrose agar, suggestive of budding mycelium. × 1100.

Fig. 3. Yeast-like growth on wort agar. × 1100.

Fig. 4. Ascus showing ascospores. × 1100.

Fig. 5. Raquet mycelium. × 1100.

Figs. 6 and 7. Heterogamous copulation. × 1100.

Fig. 8. Perhaps young ascus before spore formation, showing fusion nucleus. × 1100.

Fig. 9. Heterogamous copulation. × 1100.

Fig. 10. Perhaps earlier stage before nuclear fusion. × 1100.

Fig. 11. Terminal hypnospore. × 1100.

Fig. 12. Portion of hypha showing endospores. × 1100.

Fig. 13. Type of mycelium. × 1100.

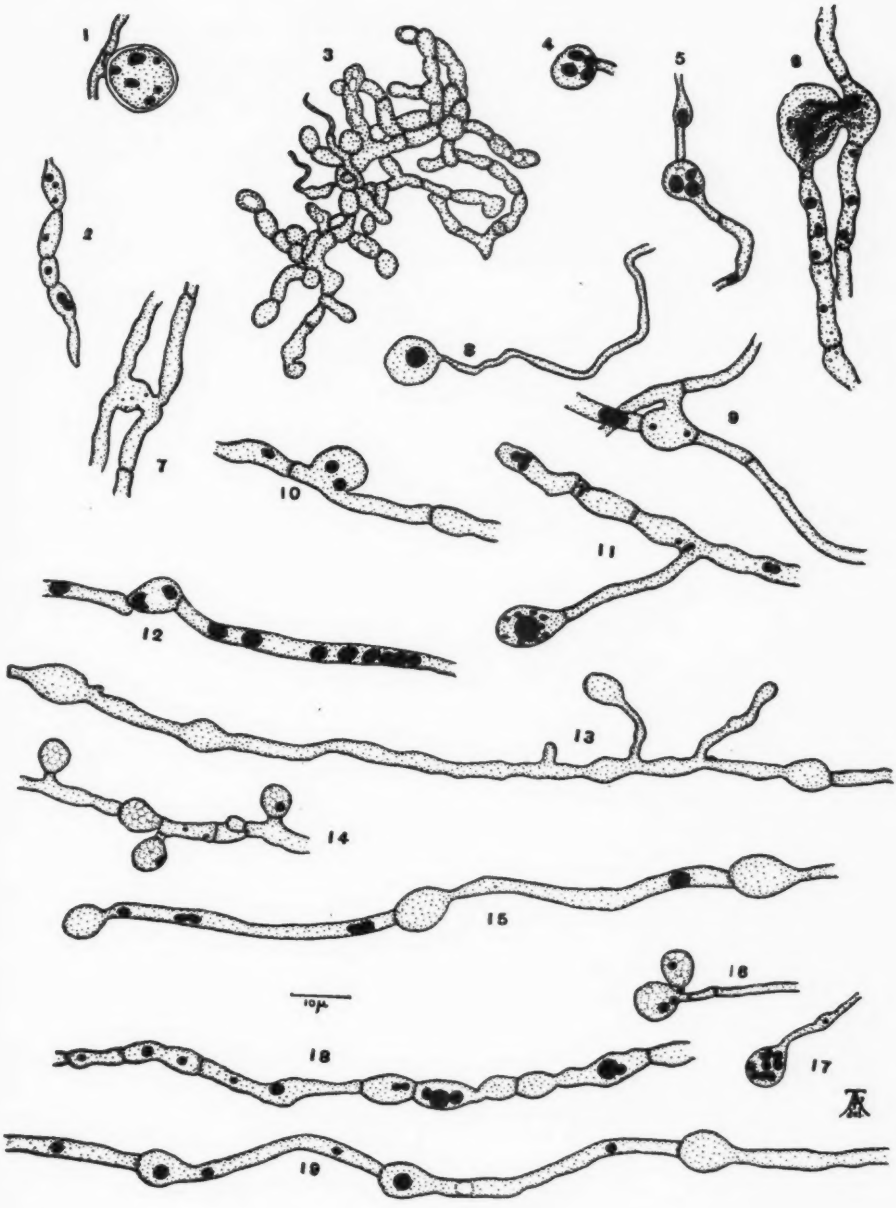
Fig. 14. Conidia. × 1100.

Fig. 15. Type of mycelium. × 1100.

Fig. 16. Conidia. × 1100

Fig. 17. Probably an ascus. × 1100

Figs. 18 and 19. Types of mycelium. × 1100.

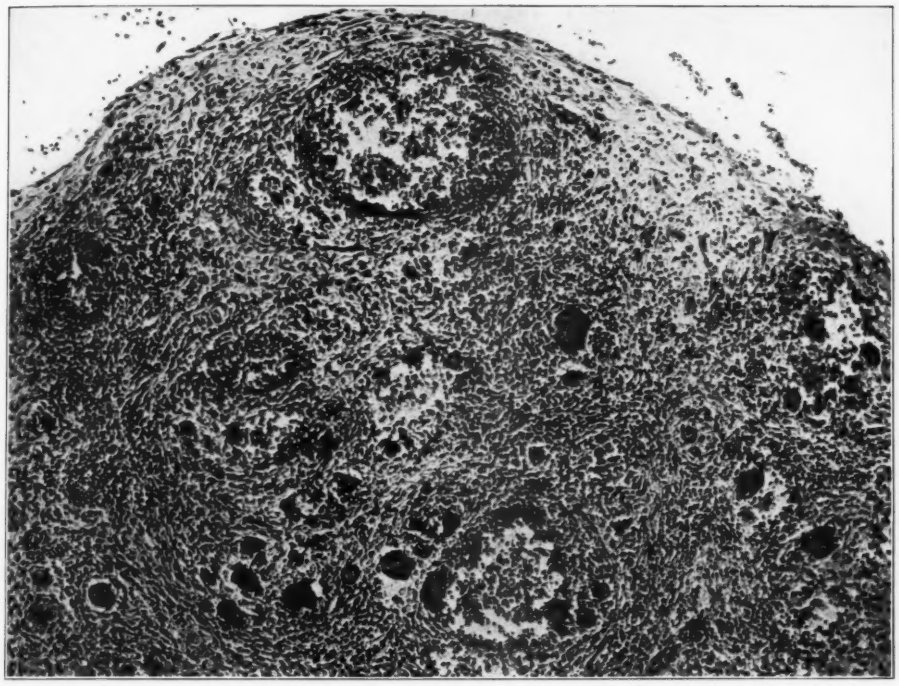


Rewbridge, Dodge and Ayers Meningitis Due to Endomyces Capsulatus

Plate 72

Fig. 20. Photomicrograph of a section through a pial nodule showing an exudate consisting of foreign body giant cells and endothelial leucocytes. There are several areas of necrosis in which are found many polymorphonuclear leucocytes. × 100.

Fig. 21. Photomicrograph showing an area of necrosis in which are found many polymorphonuclear and fewer endothelial leucocytes. Several foreign body giant cells border the necrotic area. × 300.



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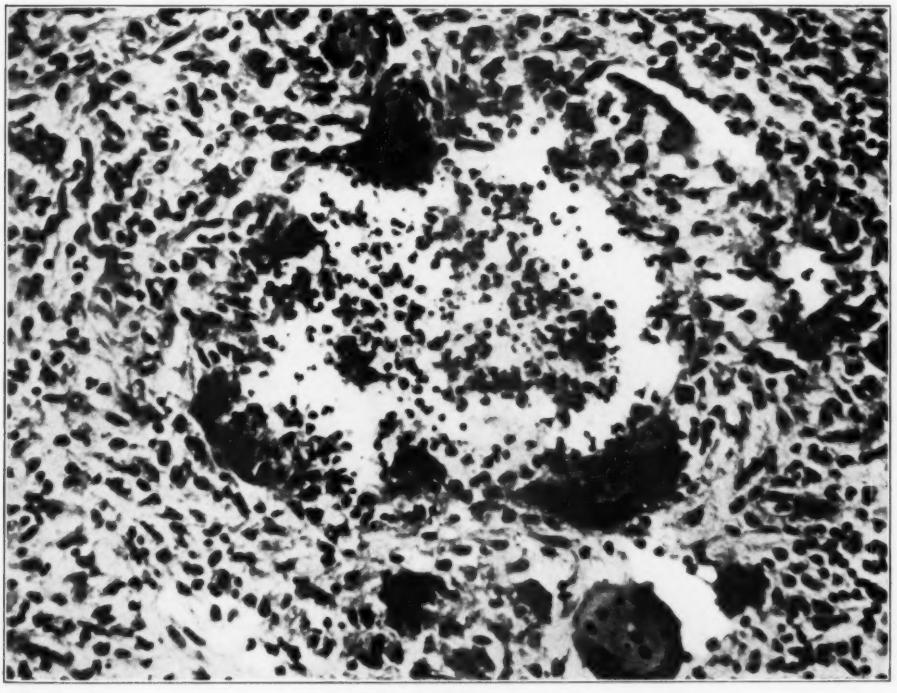

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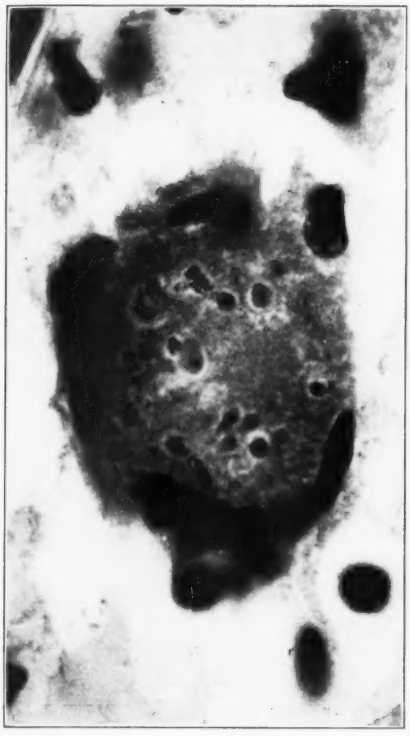
Rewbridge, Dodge and Ayers Meningitis Due to Endomyces Capsulatus

Plate 73

Fig. 22. In the cytoplasm of the foreign body giant cell are round yeast-like organisms surrounded by a clear non-staining capsule. × 1500.

Fig. 23. Three budding organisms are present in the cytoplasm of the foreign body giant cell. × 1500.

Fig. 24. Photomicrograph showing an early stage of budding which consists of a slight protoplasmic bulge at the periphery of the organism accompanied by a corresponding bulge of the capsule. × 1500.



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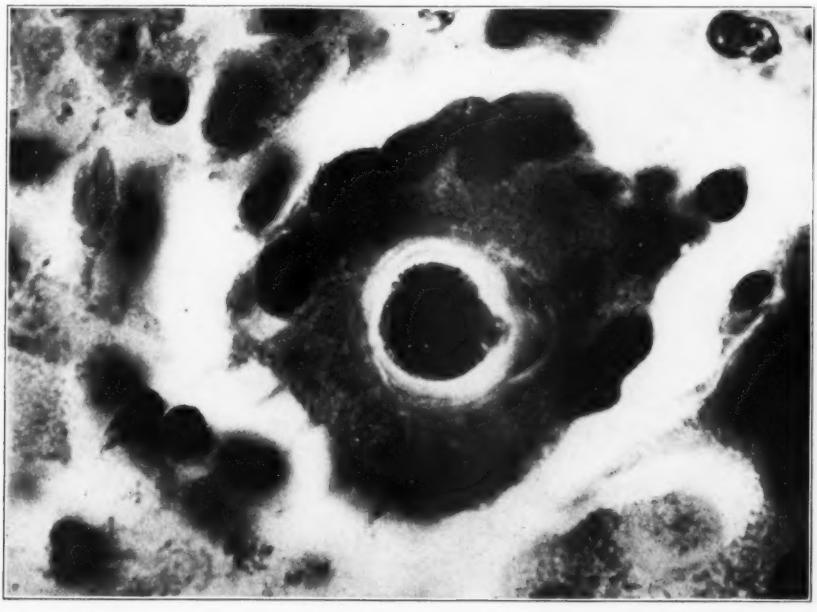

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Rewbridge, Dodge and Ayers Meningitis Due to Endomyces Capsulatus

LIGHT AS A FACTOR IN THE IMPREGNATION OF BRAIN SECTIONS WITH THE AMMONIACAL SILVER SALTS*

NATHAN CHANDLER FOOT

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While performing the experiments detailed in a recent paper [1] it was noticed that successful impregnation of the oligodendroglia, microglia and, to a lesser extent, of the neurons appeared to be largely a matter of luck; sometimes they took the silver, sometimes they did not. Already noted and commented upon by other observers, this capricious behavior on the part of these cells has not yet been satisfactorily explained. Good results were generally obtained if the sections were bromurated and impregnated immediately after they were cut; if, however, they were left in their phials in Cajal's formalin bromid for a week or more their oligodendroglia and microglia became increasingly refractory to impregnation and were soon quite undemonstrable.

There might be a number of reasons for this: the formalin might have become acid, the stronger bromid content of the modified Cajal's fixative (6 per cent instead of 3 per cent NH_4Br) used in my experiments might have been to blame, or something might have been dissolved out of these cells by the storage fluid. All these factors could, however, be ruled out: the formalin was kept neutral by the occasional addition of a few drops of 1 per cent Na_2CO_2, the original Cajal's fixative was used in parallel with the modified fluid as a control, ordinary neutral formalin was tried out as a storing fluid, but the sections remained refractory. That anything had been leached out of them seemed improbable, for the blocks from which they were cut had been in formalin for several months and fresh sections from these always gave beautiful results with the oligodendroglia if impregnated immediately after they were cut.

There remained but one readily conceivable factor to be considered: the action of daylight. It was a fact that the best impregna-

* Received for publication April 24, 1929.

tions had been made during periods of smoky or rainy weather, when the laboratory was so gloomy as to require artificial light; it was also obvious that the sections that had become refractory had been kept on a table in a window where there was often bright sunlight. In order to decide the question the following simple experiment was performed.

Experimental Work

A batch of frozen sections was cut from a brain known to contain an abundance of oligodendroglia and microglia in a good state of preservation (Brain 1 of the series described in the previous paper [1] and taken at necropsy one hour postmortem from an elderly woman). The sections were then divided into two lots: Set L was stored in formalin bromid in a clear glass bottle and placed directly in front of the window where it would get plenty of light; Set D was stored in the same fluid in a brown glass bottle and shut up in a reasonably light-tight cupboard. The two lots of sections stayed in their respective places for about ten days, during which time there was usually bright diffuse light in the window and, on a few days, brilliant sunlight. One very dark day the L-set was exposed to the focussed beam of an arc light for a few minutes, a beam not powerful enough to heat the fluid in the bottle. At the end of the ten-day period a number of sections were taken from each lot and bromurated by twenty-four hours' immersion in 4 per cent NH_4OH at room temperature followed by eight hours in 10 per cent HBr at 38°C in an incubator. While in ammonia the D-set was kept in the dark cupboard, the L-set in the window; while in HBr, both sets were of necessity kept in the dark, as the incubator had no glass door.

Both lots were then impregnated respectively with silver diammino hydroxid and silver diammino carbonate, the complete technic for the procedure is given in the preceding paper.[1] It was at once evident that the two lots behaved quite differently. Even before impregnation it was thought that the D-set had a yellower tinge and that the ammonia through which it had passed remained clearer than that in which the L-set had been exposed to light and in which there was a certain milky opalescence. After impregnation it was seen that the D-set became more intensely stained in both silver solutions, that it gave off clouds of a blackish precipitate in the buffered formalin developer (whereas the L-set did not) and that the

difference in the two sets treated with the carbonate was more noticeable than that in those treated with the hydroxid.

In the carbonate the D-set became fox-red, with a browner medullary portion; the L-set became seal-brown and more diffusely impregnated, with less distinction between the gray and the white matter. At this stage, the difference was so marked that the two sets might be readily sorted out, the one from the other. In the developer they became more alike in their gross appearance and could no longer be readily distinguished, although the D-set was slightly darker than the L-set. In the gold toning bath the D-set grew more bluish violet while the L-set turned a brownish violet; hypo merely accentuated this slight difference.

The final test came under the microscope, after the sections had been counterstained and mounted. It was seen that the D-sections were somewhat more distinctly impregnated with silver diammino hydroxid than were those of the L-set and the neurons were much more distinctly defined; otherwise there was but little choice between them.

The sections impregnated in silver diammino carbonate, however, were strikingly different; there was apt to be troublesome precipitate in the L-set and the impregnation of the oligodendroglia and microglia was decidedly poor, or altogether lacking. Liesegang[2] would probably explain the precipitate by the production of false "reduction centers" (Silberkeime) by the action of light. Of the twenty-four D-sections impregnated in three separate groups in as many separate solutions of the carbonate (in order to rule out differences in making up the carbonate baths) every section of the set showed good impregnation of the oligodendroglia, while four gave unusually brilliant results; there was not a single failure. Of the fifteen sections in the L-set, on the other hand, impregnated in the same solutions at the same time and in the same manner as the others, seven utterly failed to demonstrate the oligodendroglia, two showed but indistinct shadowy forms of these, four impregnated them very slightly, and only two caught them at all well. In the last instance the processes were poorly impregnated, interrupted or stippled. In other words, to put the most charitable construction on the results, the D-set showed one hundred per cent of "takes" in the case of the oligodendroglia, while the L-set showed sixty per cent partial to complete failures and forty per cent quite worthless.

In order to collect further data on the effect of light upon sections to be impregnated with silver diammino carbonate, the same lots of sections used in the preceding experiment were kept in their respective bottles (one in the light of the window and the other in the dark cupboard) until a month had elapsed. They were then impregnated with silver diammino carbonate in the same manner as those of the ten-day set, eleven slides being prepared from each lot. Under the microscope it was found that the results were quite similar to those just detailed; the light-exposed sections were strikingly inferior to those kept in the dark. Although the oligodendroglia was no longer as precisely impregnated as it was in freshly cut sections or the ten-day D-set, it was readily recognizable in every one from the thirty-one-day D-set; nothing better than the faintest suggestion of cytoplasm and processes could be obtained in the case of the one-month L-set. The results were essentially one hundred per cent fair to good impregnation in the D-set, one hundred per cent poor to worthless in the L-set. Furthermore, there was such an increase in precipitation in the latter as to render it practically useless for this reason alone. It was evident, even when sections were kept in a brown bottle in the dark, that the impregnation of the oligodendroglia became less and less precise as time elapsed between sectioning and impregnating. As before, the astrocytes remained relatively unaffected by long exposure to light, while the nerve cells occupied a position midway between these and the oligodendroglia in respect to their light sensitivity.

While this is, of course, too small a series for decent statistical purposes, the result is so clean-cut that the deduction is sufficiently obvious: light distinctly affects the oligodendroglia. Added proof may be adduced from the result of observations made in many untabulated series of unsuccessful impregnations performed on sections kept in the light, during the carrying out of the experiments described in the paper already quoted. That one or two sections of the L-set should show some impregnation of these cells is readily explained by their having become folded or bunched in the fixative, one overlying the other. Some of them were thus partially shielded from the light, while others received its full force. This assumption is further borne out by the fact that those sections in the L-set that did show impregnation of the oligodendroglia were distinctly "spotty"; there were whole fields with no satisfactorily impregnated cells and then

there would be several where they showed partial or complete impregnation. Seven sections of the fifteen, be it remembered, showed no impregnation of the cytoplasm or processes of these cells, while every one of the twenty-four D-sections showed satisfactory or beautiful impregnation.

The microglia cells seemed to share this idiosyncrasy for light, but the results in their case were less striking, probably because the technic was tempered to the oligodendroglia rather than to the microglia, which requires stronger solutions of the silver salts for satisfactory impregnation.

Speculation as to what happens to the cytoplasm of the oligodendroglia under the influence of light would be rather idle at this stage of the investigation; the fact remains that, through its action, these cells distinctly lose their capacity for reducing silver diammino carbonate to metallic silver. As the sets impregnated with silver diammino hydroxid showed somewhat better impregnation in the case of the D-set as compared with the L-set, we may infer as a general rule that one should store frozen sections of brain in brown, rather than in clear glass bottles, and in the dark rather than exposed to light. Apparently light does not permeate blocks of tissue very deeply, for the material used in this experiment had been kept in formalin in the window, exposed to all the light there was; one need not, therefore, be as careful of opaque blocks of tissue as one should be of translucent sections of brain. Obviously, caution would dictate the dark storage of brain tissue in preference to keeping it in the light. There has been a good deal of discussion as to whether impregnation should be performed in the light or in the dark, but I do not recall ever seeing any mention of the effect of light upon brain sections *before* their treatment with light-sensitive silver salts.

Summary and Conclusions

1. Frozen sections of brain tissue should be shielded from light before and up to the time that they are impregnated with silver salts.

2. Light inhibits the impregnation of the oligodendroglia and microglia with silver diammino carbonate and produces troublesome precipitates in the sections.

3. It does not appear to influence the impregnation of the macroglia materially.

4. It somewhat inhibits the impregnation of the cortical neurons with silver diammino hydroxid, after bromuration. This fact may be made use of by technicians desiring to suppress these cells and their dendrites and to accentuate the microglia by contrast.

REFERENCES

1. Foot, N. C. Comments on the impregnation of neuroglia with ammoniacal silver salts. *Am. J. Path.*, 1929, v, 223.

2. Liesegang, R. E. Die Kolloidchemie der histologischen Silberfärbungen. *Kolloidchem. Beihefte*, 1911, iii, 1.

MULTIPLE FOCAL SPLENITIS OF GUINEA PIGS*

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The universal use of the guinea pig in laboratory experiments pertaining to bacteriology makes it significant to recognize and understand the spontaneous diseases of this animal. The predilection of *Myobacterium tuberculosis* and *Bacterium tularense* for the spleen following their experimental injection into the body of guinea pigs makes the recognition of spontaneous infection of this organ imperative if one is to avoid errors in the interpretation of splenic lesions following injections.

During the last year I have observed a multiple focal type of splenitis in guinea pigs which, so far as I have been able to ascertain, has not heretofore been described. The disease was encountered largely among the unused or stock animals which were subjected to necropsy examination as a routine following spontaneous death. In three instances material was secured through the kindness of others who recognized the lesions in animals that had died following the injection of various pathogenic bacteria. Twenty-one cases of the disease were distributed throughout the year 1928 as follows: February, two cases; June, two cases; July, three cases; August, one case; September, two cases; October, nine cases; November, one case; and December, one case. Although only twenty-one cases were actually studied, it is quite likely that other cases occurred, since animals, that had died spontaneously and whose bodies were in an advanced stage of putrefaction were not examined. Data were not obtainable on the large number of guinea pigs from the stock pens which were used experimentally by various workers.

PATHOLOGIC CHANGES

Recognizable symptoms were not observed in any of the cases studied. A large stock of animals is maintained under the observation of the attendant throughout the day, and sick or suspiciously

* Received for publication May 6, 1929.

inactive animals are segregated at once. Most of the material studied was obtained from animals that apparently had died suddenly, or during the night. Dead animals in which this condition has been found had usually been well nourished, and generalized demonstrable effects of a disease process were absent.

Grossly the disease appears to be confined to the spleen which is usually noticeably enlarged and striking in appearance, owing to the presence of the large number of lesions. In the material examined, the lesions were of two general types. In eleven cases the spleen possessed enormous numbers of small grayish white foci, seldom measuring more than 0.1 cm. in diameter and usually much less (Fig. 1). These projected slightly above the surface and were found diffusely distributed throughout the body of the organ. In the remaining ten cases the lesions were more variable in size, being from 0.1 to 0.4 cm. in diameter and of a yellowish color. The surface lesions were less numerous, varying from a few to less than twelve in the respective spleens. In a few instances enormously enlarged spleens were observed without gross focal lesions, although sections examined microscopically revealed pathologic changes similar to those characterizing the spleens possessing gross alterations.

The lesions were essentially those of focal necrosis, and a study of the respective spleens in which the disease was spontaneously present revealed all gradations of the necrobiotic process (Figs. 1, 2 and 3). The majority of the lesions appeared to have had their inception in the splenic nodules, the normal elements of which become obliterated as the disease progresses. The earlier changes seem to be those of coagulation necrosis, while in the later stages the center of the lesions shows a marked tendency to caseate. As necrosis proceeds, polymorphonuclear and mononuclear leukocytes appear at the periphery of the lesion, and these cells eventually replace much of the necrotic substance. In the more advanced lesions the majority of the cells in the zone between the necrotic center and the periphery undergo striking retrogression and become clear, with nuclear elements not discernible. The more chronic lesions become definitely circumscribed by a fibrous connective tissue capsule of variable thickness. In many of the lesions this fibrous structure had so fused as to present a hyaline appearance (Fig. 4).

Smears made from the purulent material of the lesions and sections stained by gram's method revealed moderate numbers of gram

positive streptococci. Many sections were stained with carbol-fuchsin in an attempt to demonstrate the presence of acid-fast bacilli, with negative results.

Experimental Methods

Attempts were made to isolate the etiologic agent and to produce the disease experimentally. Many of the cases were unsuited for this phase of the investigation due to advanced postmortem changes, and, as a consequence, only thirteen of the cases were so utilized. The spleen was removed as aseptically as possible under the circumstances, and a portion was macerated in sterile sodium chloride solution. The resultant emulsion was then strained through several thicknesses of sterile cheesecloth and at once inoculated into apparently normal guinea pigs from the stock supply from which the occasional cases of the disease were appearing. In most cases three animals were used, which were inoculated as follows: one received 1 cc. of the emulsion intraperitoneally, one received 1 cc. of the emulsion subcutaneously, and one received 2 cc. of the emulsion by mouth.

Cultures were not attempted from many of the experimental animals that died, because of advanced putrefactive changes. Of the thirty-three guinea pigs inoculated, twenty-five died at intervals of two to one hundred ninety-two days subsequent to inoculation, and eight were killed at the termination of the experiment. The eight that were killed had been inoculated at intervals of ninety-three to two hundred nine days previously. From the data obtained from the inoculation experiments several facts seem apparent, the most important of which are:

1. In seven instances splenic lesions simulating those of the spontaneous lesions were found grossly or microscopically.

2. The microörganism most frequently encountered in the animals that died subsequent to inoculation was a gram positive streptococcus. Many of the strains were hemolytic in character.

3. Purulent peritonitis was often induced by the intraperitoneal and subcutaneous injection of the emulsified inoculum.

4. In only one instance in which the material was introduced orally were there abdominal lesions. This animal, which died after one hundred nine days, revealed multiple abscesses in the walls

of the intestinal tract, and gram positive streptococci were obtained from the lesions. Although the spleen was slightly enlarged, gross lesions were absent; microscopically typical chronic lesions of focal splenitis were present.

5. Much variation seemed to exist in the pathogenicity of the infective material and in the susceptibility of the inoculated animals.

6. The evidence suggests a non-specific gram positive pyogenic streptococcus as a possible etiologic agent in this condition.

7. The exact portal of entry of the organisms responsible for the disease is obscure.

Thinking that there might be some correlation in the occurrence of the spontaneous focal splenitis described and the not uncommon disease of guinea pigs known as "epizoötic lymphadenitis," "lumps," "epidemic lymphadenitis" and "purulent adenitis" described by Boxmeyer,[1] Holman,[2] Hardenbergh,[3] Meyer,[4] Megrail and Hoyt,[5] necropsy was performed on sixty-five animals affected with purulent adenitis. The lot consisted of thirty males and thirty-five females. In eighteen cases the spleen was enlarged, but in none were there gross lesions comparable with those encountered in the spontaneous cases of focal splenitis. In five of the eighteen enlarged spleens, microscopic alterations typical of the chronic type of focal splenitis were observed. The incidence of focal splenitis was highest in October, 1928. In December, the necropsies were performed in which enlarged spleens were found in the eighteen animals. The possibility that the focal splenitis and the epizoötic lymphadenitis existed simultaneously and independently is worthy of consideration. In support of the hypothesis that the diseases are separate entities are the necropsy data in thirty-one of the guinea pigs affected with purulent adenitis which were examined in April, 1929. In none of these was the spleen enlarged. This seems significant in view of the fact that cases of multiple focal splenitis had not been observed since the latter part of December, 1928.

Comment

According to Meyer, the spleen in the disease of guinea pigs designated as pseudotuberculosis, due to a gram negative bipolar rod, is studded with whitish nodules. However, in pseudotuberculosis "the enlarged mesenteric, iliac, periaortic and occasionally inguinal

lymph nodes are transformed into a mass of thick yellowish pus and fibrous tissue." Abscesses are also present in the liver and may involve the kidneys and uterus. In the twenty-one animals from which material was obtained for this study the liver was not affected. The gross lesions in fact were confined entirely to the spleen. The pathologic anatomy and the bacteriologic data seem sufficient to designate the two conditions as separate diseases.

One must be cautious in interpreting the microscopic lesions in the spleen of the seven inoculated animals since it must be borne in mind that the animals used for the inoculations were from the same stock in which the spontaneous cases were appearing. For this reason the assumption that the splenic lesions which were found in the experimental animals had their etiologic inception from the inoculated material, does not seem warranted. Until lesions of the disease can be induced with some regularity by the adherence to a given procedure, it would seem presumptuous to claim that the specific lesions have been produced experimentally.

As further material is encountered it is proposed to make a detailed bacteriologic report of the microörganisms isolated.

Summary

Twenty-one cases of a spontaneous disease of guinea pigs which is characterized by a multiple focal type of splenitis, were studied. Morphologically the disease seems to be confined to the spleen and microscopically the lesions are those of focal necrosis. In an attempt to produce the condition experimentally, thirty-three guinea pigs were inoculated with emulsions of the lesions from thirteen of the cases. Twenty-five of the experimental animals died; the most frequent lesions were those of purulent or fibrinous peritonitis. From many of the inoculated animals which died, a gram positive streptococcus was obtained. The spleens of a few of the experimental animals possessed microscopic lesions similar to those observed spontaneously. The impossibility of ruling out spontaneous cases of the disease in the experimental animals makes it unwise to assume that the specific splenic lesions have been produced experimentally. For the same reason there does not seem to be any correlation between the condition known as purulent adenitis and the splenic disease described.

REFERENCES

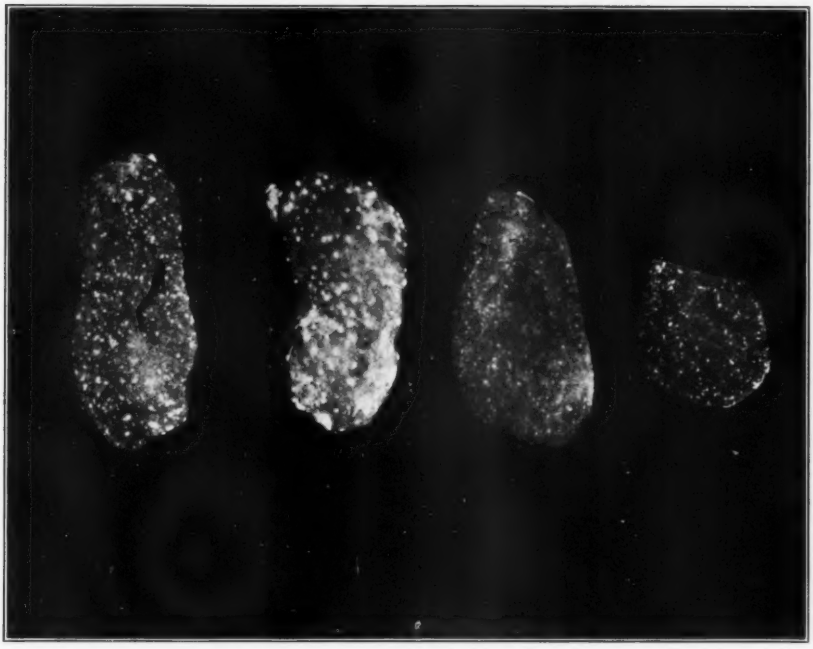
1. Boxmeyer, C. H. Epizootic lymphadenitis: A new disease of guinea pigs. *J. Infect. Dis.*, 1907, iv, 657.
2. Holman, W. L. Spontaneous infection in the guinea-pig. *J. Med. Res.*, 1916, xxxv, 151.
3. Hardenbergh, J. G. Epidemic lymphadenitis with formation of abscess in guinea pigs due to infection with hemolytic streptococcus. *J. Lab. & Clin. Med.*, 1926, xii, 119.
4. Meyer, K. F. Communicable Diseases of Laboratory Animals. In: The Newer Knowledge of Bacteriology and Immunology. University of Chicago Press, 1928, 607.
5. Megrail, Emerson, and Hoyt, R. M. Epizootic lymphadenitis in guinea-pigs. *J. Infect. Dis.*, 1929, xliv, 243.

DESCRIPTION OF PLATES

PLATE 74

FIG. 1. Spontaneous splenitis. The multiple miliary character of the lesions is apparent.

FIG. 2. Spontaneous splenitis. An early lesion with beginning necrosis. A peripheral fibrous zone has not developed. × 125.



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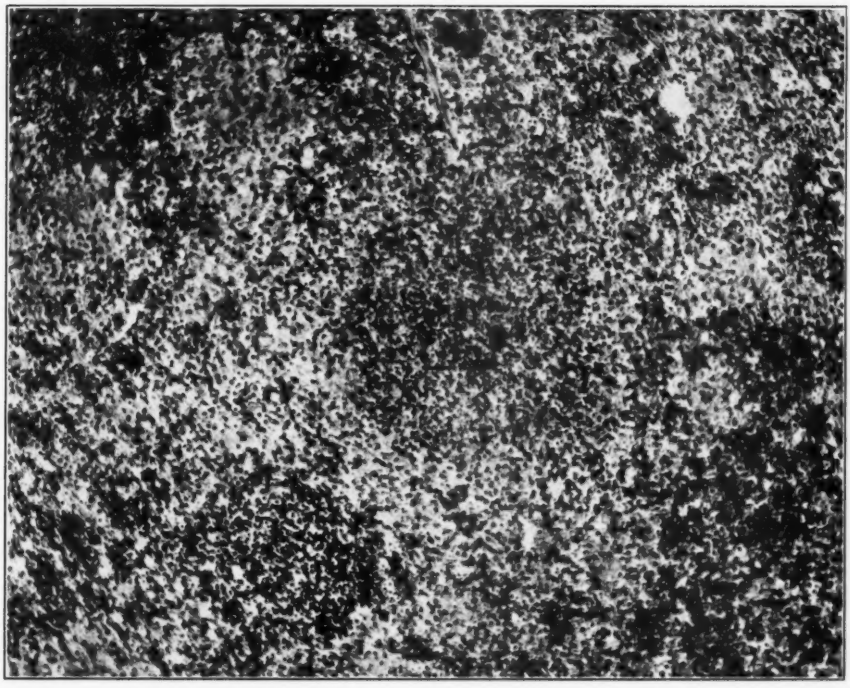


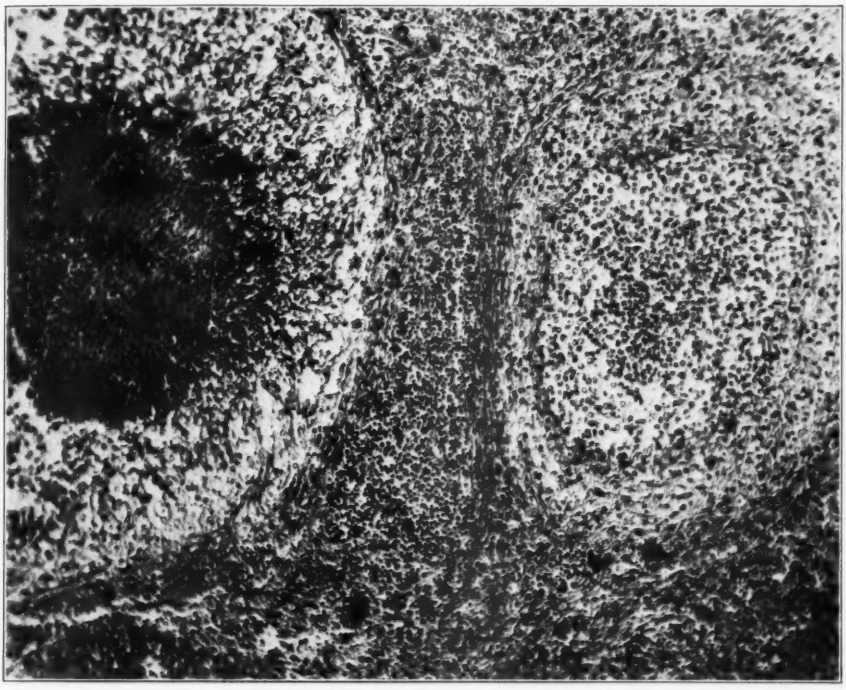
2

Feldman Multiple Focal Splenitis of Guinea Pigs

PLATE 75

FIG. 3. Spontaneous splenitis. Multiple lesions of somewhat different character. The one on the left possesses a necrotic center; the cellular integrity of that on the right is still intact. × 150.

FIG. 4. Spontaneous splenitis. The details of a typical lesion are shown. The broad zone of encapsulating cells at the periphery is characteristic. This has a tendency to undergo a hyaline change as the lesion progresses. × 180.



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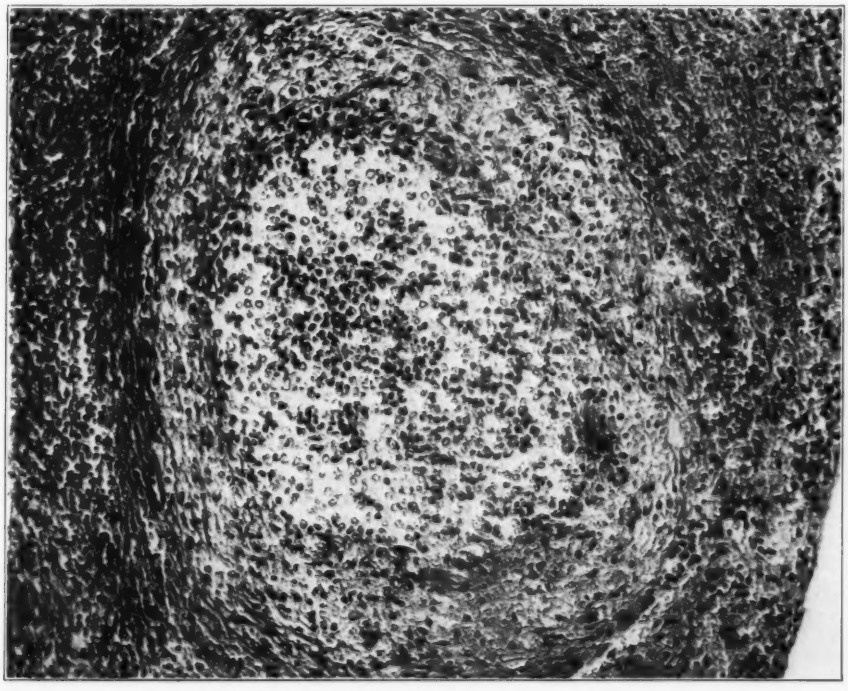

4

Feldman Multiple Focal Splenitis of Guinea Pigs

THE DIAGNOSTIC RELIABILITY OF FROZEN SECTIONS*

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The words "frozen sections" have been heard for many years by some pathologists with a certain feeling of reluctance, distrust, or indifference. Certain pathologists who have not had a large personal experience with all methods of preparation of tissues frequently have approached the subject rather apologetically. Despite this attitude of mind, the method of making frozen sections is at least twenty-three years old, and its reliability has been well tried out here on 208,255 surgical specimens including 28,833 carcinomas.

There are several reasons why some pathologists have failed to realize the value of frozen sections. Such sections are made to best advantage from perfectly fresh, unfixed tissues. Their application to necropsy material or to material which has stood longer than half an hour is not very satisfactory. It has been difficult to obtain good stains; the best is Unna's polychrome methylene blue (Gruebler's) or Terry's recent modification of this stain. Before the World War there was no difficulty in obtaining good stain. During and after the War, stain was made in the laboratories of the clinic; for the last three years Terry's modification of this stain has been used. The stain does not make the diagnosis; it merely saves time in finding the cells. A well trained cytologist could make the diagnosis without stain.

It must be remembered that most pathologists see very little fresh tissue. They are quite naturally and justly wedded to paraffin and celloidin sections. With the material they have had, their technique has been admirable but their studies have been limited largely to the low powers of the microscope. Perfectly fresh unfixed tissues, on the other hand, may and should be studied with oil immersion lenses.

There are many tests of the diagnostic and prognostic reliability both of unfixed and of fixed frozen sections: Let us suppose, for example, that a surgeon removes a lymph node from the region of the

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cecum or sigmoid and the report, based on a fresh, unfixed frozen section, is that the tissue is malignant. If the condition in the resected cecum turns out to be tuberculosis or the condition in the sigmoid, diverticulitis, how long do you think the surgeon would keep his pathologist? Suppose the surgeon removed a lymph node from the neck and the pathologist reported it to be tissue from a case of Hodgkin's disease or of lymphosarcoma and the lymph nodes were excised and found to be tuberculous; again, how long would the pathologist hold his position? If a specimen obtained by biopsy from a bone tumor were reported to be from malignant tissue and the condition were, after amputation, found to be chronic osteomyelitis, or perhaps syphilis, how long would the surgeon continue to have faith in the pathologist's ability? Suppose the surgeon curetted a uterus and the curetted specimen was reported to be from a malignant growth, and on hysterectomy nothing but a polyp or an hypertrophic endometrium was found, how good would the pathologist's reputation be in the minds of his clinical confreres?

It happens occasionally that a pathological diagnosis that is negative for malignancy is followed by the removal of malignant tissue, but this is no fault of the pathologist since he can examine only that tissue which is given to him and it may not contain anything that is malignant.

Frozen sections of fresh, unfixed tissue are so reliable in the hands of a well trained pathologist that he can make the diagnosis of a malignant condition, correctly, from a single cell. This may seem impossible; in fact a well known pathologist with very little experience with fresh tissue told me that it could not be done. The correctness of the statement that it can be and is being done has been proved. Without the pathologist having any knowledge of the specimen or the patient, a colleague, assistant, or technician may place a single cell under the oil immersion lens and may ask the pathologist to make a diagnosis. Let us suppose the cell is in a peripheral sinus of a lymph node, as frequently occurs. Such nodes are often removed so that the surgeon may know whether or not a mass is malignant and just how extensive it is if it is malignant. The report is that the condition is malignant and the surgeon resects the mass; the resected specimen must show the carcinoma if the original report were correct. In my own experience it has never failed. There are two more examples out of many which might be related. Sup-

pose the test specimen is from the uterus and the report is that the tissue is malignant. I have never seen a benign uterus removed after such a report. Suppose it was from a bone tumor and an amputation followed. I have never seen an amputated leg or arm, or any portion of these, that did not show malignancy to be present after such a cellular diagnosis.

There is another test of the accuracy of the method which unfortunately requires years to carry out. The cytologic method of diagnosis has been used for more than eleven years, and hence there has been enough time to carry it out in many cases. Let us suppose that one, or a few, of the malignant cells are found in a case of chronic mastitis or in a chronic gastric ulcer and are recorded as being malignant and the subsequent histories of the patients are followed. What might be expected? Some of the patients would be cured, or at least would be alive for more than eleven years, and we would not know whether the pathologic diagnosis was correct or not; but some of these patients actually return with recurrence of the malignant condition despite radical treatment. In a study of frozen fresh tissue comprising eight hundred sixty-nine freshly resected or excised gastric ulcers, 4.4 per cent contained the "malignant cell" inside of the gastric tubule and 5.2 per cent, inside, or just outside in the immediate stroma. Seven and five-tenths per cent of the first group of patients are dead of unknown causes and 15.2 per cent of the second group are known to be dead of carcinoma within the eleven-year period of study.

There are many other instances of the subsequent or postoperative history revealing the reliability of diagnosis by means of study of fresh, unfixed and fixed, frozen tissue. I bring the subject to the attention of pathologists merely to emphasize three points:

1. Surgeons and other clinicians know the value of frozen sections and have depended heretofore on professors of pathology to furnish candidates for positions in surgical pathology. Such surgeons are going ahead with the use of frozen sections despite the apathy of many general pathologists and are going to develop their own pathologists who may sooner or later become divorced, unfortunately, from the field of general pathology.

2. Pathologists, if interested at all in tissues, are missing a great opportunity to see cells as they are; they are failing to improve their understanding of their own subject.

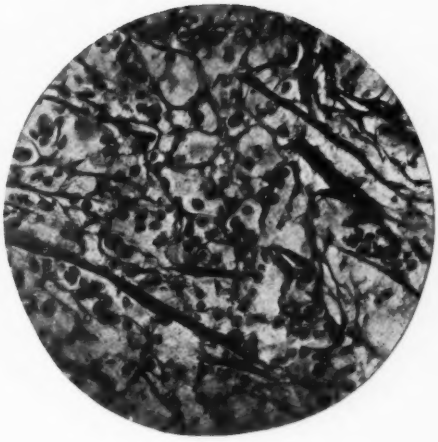
3. By their lack of clinical interest and failure to utilize fresh tissues they are rapidly becoming thought to be less efficient as microscopic diagnosticians.

My plea is that pathologists open their eyes to a method which will startle them after a little practice. "I have never seen such beautiful cells" is the usual comment when properly made frozen sections are demonstrated to pathologists who are accustomed to seeing only embedded material.

DESCRIPTION OF PLATES

PLATE 76

Fresh unfixed and formalin-fixed frozen sections.



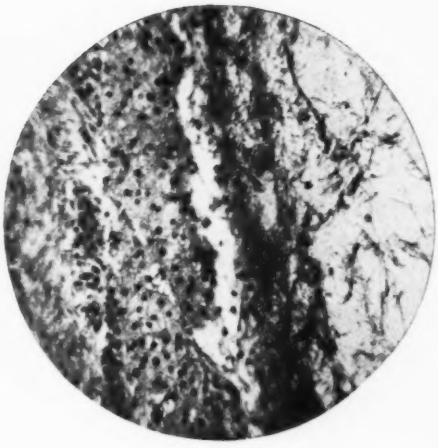


PLATE 77

Fresh unfixed and formalin-fixed sections.

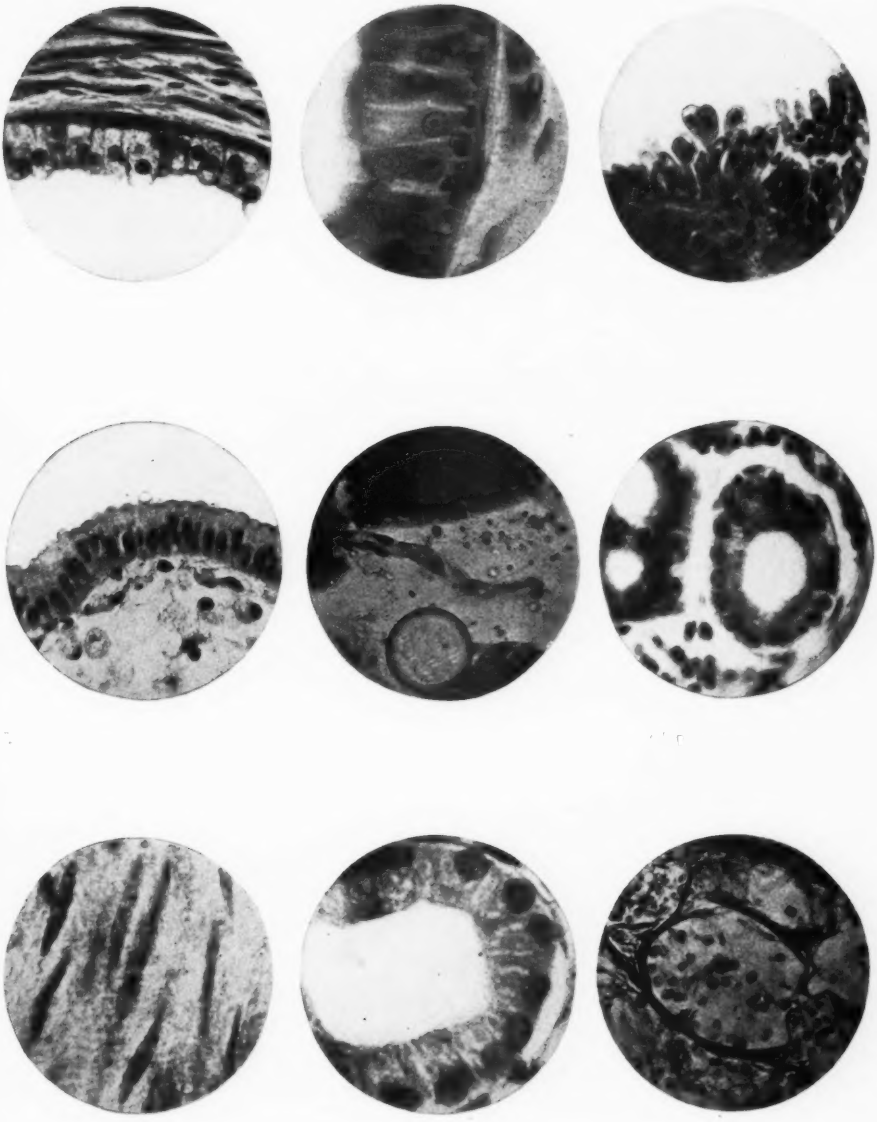
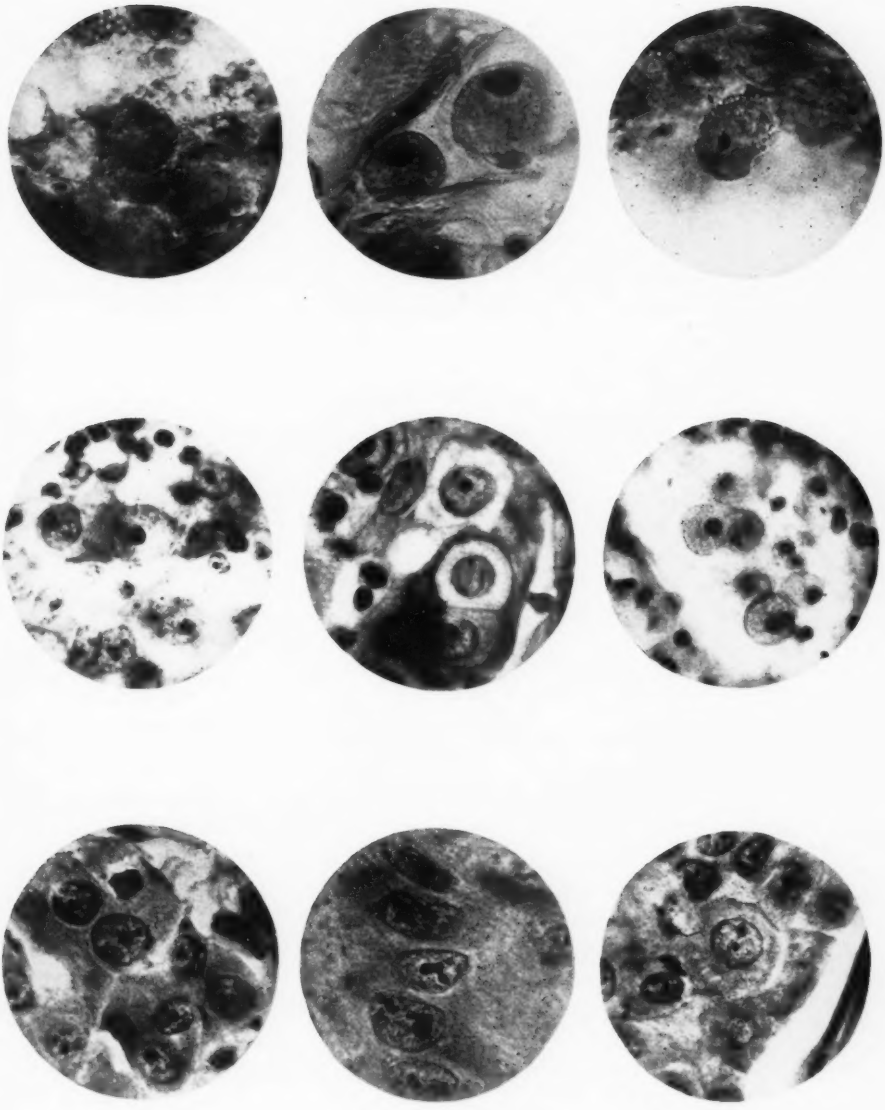


PLATE 78

Malignant cells from fresh unfixed and formalin-fixed frozen sections.



THE PATHOLOGY OF HEMANGIOMA OF BONE *

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The number of cases of hemangioma of bone described in the literature is rather small and the amount of pathological detail remarkably scanty. It is for this reason that we are undertaking here to describe the findings in the seven cases which we have been fortunate enough to study although we are well aware of the limitations of our material. In most of the instances only one slide stained with the usual hematoxylin and eosin is available and in the other cases the amount of tissue removed at operation is very small, usually only a biopsy having been performed. As the clinical histories and roentgen findings have been given completely in a recent article (Bucy and Capp [1]) we shall limit ourselves here to the presentation of the cases in a tabular form (Table I) and the pathological findings will be presented as a general discussion rather than detailed description of the individual case.

Clinically hemangioma of bone is rare. In all we have been able to collect from the literature and our own cases only thirty-nine in which symptoms relative to it were present. Thirteen of these were located in the skull, twelve in the vertebrae and fourteen in other bones of the body, no one of which showed any predominance in frequency of involvement.

On the other hand, Töpfer [2] has recently demonstrated that hemangioma of the vertebrae is a common pathological finding. By careful routine studies in which he sectioned the vertebral column of 2,154 bodies at autopsy, he demonstrated that hemangiomas were present in 257 cases or 11.93 per cent. These varied greatly in size, from a pea up to complete involvement of the entire vertebral body. But in no case was there any history of symptoms referable to the lesion. Doubtless if similar studies of the other bones of the body

* Four of the seven cases included in our study were kindly furnished from the Bone Sarcoma Registry of the American College of Surgeons.

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were made many small hemangiomas would be found lying dormant within the bone.

As will be noted in Table I, as well as in the cases reported by other authors, females are a little more often affected than males, about in the ratio of 55 to 45. There seems to be a definite increase in frequency during the fourth and fifth decades of life although hemangiomas of bone have been found to be congenitally present in the new born (Stamm [3]) and have been found in the bodies of individuals 90 years of age (Makrycostas [4]).

In many cases multiple lesions exist involving either other bones of the body or some of the internal organs. Such cases have been described, especially by Brandt [8] and Stamm. The liver is the organ most frequently involved by multiple hemangiomas. In the case of hemangioma of the vertebra several vertebral bodies are often affected simultaneously, 13.2 per cent of the cases in Töpfer's series.

Hitzrot [9] states that hemangiomas in the long bones usually arise near or at the epiphysis. This is in accord with our observations. However, his statement that hemangiomas arising in the cranial bones usually lie near the suture lines does not seem to be correct. In Case 4 the lesion was a little posterior to the center of the parietal bone.

Grossly the lesion is characterized by a swelling of the bone which is usually of bony hardness although in a few cases the center is soft and on examination of the patient this soft center is found to pulsate (Mauguière [10] and Zajaczkowski [11]). These few cases have occasionally been confused clinically with so-called bone-aneurysm.

If the tissue is incised at operation the hemorrhage is usually so profuse that the surgeon is unable to see the true appearance of the tumor. It can be determined only that there are numerous fine bony trabeculae which are quite friable. If such lesions are examined after removal they will be found to consist of a very vascular tissue lying between the bony trabeculae. Two other types have been mentioned. The tumor removed by Hitzrot had a cystic center which contained a straw-colored mucoid fluid. The wall of this cyst was lined by organizing blood clot beneath which lay the cavernous hemangioma. Such changes in hemangiomas of bone are apparently uncommon, but have been frequently reported in cases of hemangiomas elsewhere, especially in the cerebellum (Cushing and Bailey [12]). The other type is like that of Case 6, which was operated upon by

TABLE I

Case No.	Registered by	Registry No.	Initials	Sex	Age in years	Location	Remarks
1	P. C. B.	940	S. M.	F	62	3, 4, 5, 6 D. vert.	Compression myelitis. Laminectomy. Recovery
2	P. C. B.	941	M. L.	F	49	Rt. scapula	Biopsy. Roentgen therapy. Improvement
3	A. H. Galvin	153	Mrs. J. T. H.	F	38	Left ilium	Biopsy. Roentgen therapy. Complete recovery
4	H. Cushing *	230	J. M. C.	F	55	Left parietal	Removal. No recurrence
5	P. E. McNabb	849	C. C.	M	20	Rt. ulna	Removal. No recurrence
6	A. P. Ashhurst †	249	V. D. A.	M	35	Rt. tarsal scaphoid	Curettement. Recovery
7	D. B. Phemister ‡	...	M. McM.	F	40	Rt. clavicle	Removal. No recurrence

* Reported by Dr. Cushing[4] in 1923. † Mentioned by Dr. E. A. Codman.[6] ‡ Reported briefly by Dr. Phemister.[7]

Ashhurst, in which the tissue was not particularly vascular and had the appearance of currant jelly. These cases apparently are very uncommon. We have found no similar cases reported. Hitzrot described a type which he calls "ossifying" and of which the case of Lücke [13] is given as an example. However, ossification seems to be common to all cases of hemangioma of bone.

Of more importance is the fact that although these tumors are frequently found to have broken through the cortex of the bone, the periosteum which has become stretched over the surface of the tumor is unbroken. Cases 5 and 7 were excellent examples of this as will be seen in Fig. 1.

Microscopically the different tumors have an essentially similar structure but certain variations are seen. For the most part the tissue is composed of large cavernous spaces which contain blood cells and are lined with a layer of flat endothelium. The intervascular tissue is loose and rather acellular. The cells are usually spindle-shaped, but some are stellate. The nuclei are ovoid and contain but a small amount of diffusely scattered chromatin. The stroma of connective tissue consists of a loose network of fibers varying greatly in amount. In the tumors reproduced in Fig. 2 it is scanty, while in that shown in Fig. 3*a* it is very abundant. Occasionally large round cells with small pyknotic nuclei and vacuolated cytoplasm are seen. These cells are the so-called "foam cells," and are undoubtedly undergoing fatty degeneration. In the same areas can usually be seen cells whose cytoplasm is occupied by one or two large globules, while in the surrounding tissue, often in the center of a group of these degenerating cells, can be seen globules of fat lying loose among the fibers. In such places there are very few or no cells present. There are often a few small collections of true fat cells. We have never seen a case in which these groups were large or abundant. However, both Makrycostas and Töpfer mention cases in which there is such a large amount of adipose tissue present that the term hemangiolipoma is warranted. Ballance [14] states that in the case he examined only a small amount of lipoid tissue was present.

In Case 1, cells which we interpret as remnants of the destroyed myelogenous tissue are present. These cells have a small, rather compact deeply staining nucleus with a large, rounded cytoplasm which contains granules. By specific methods these granules take the basophilic stain in some cells and the eosinophilic in others. We

believe that these are true myelocytes. We have been unable to find any reference to the finding of such cells in the cases of hemangioma of bone previously reported.

The lining of the vascular spaces as we have previously stated is generally a single layer of flattened endothelial cells, quite comparable to those seen in normal vessels. Such is apparently the rule in these cases (Mauguière, Ballance, and Perman,[15] etc.). However, in one instance (Case 5) the cells are much thicker and the nuclei take a deeper stain. Occasionally places are seen where the lining is two or three cells in thickness instead of one. In this case we have seen no examples of proliferation of the cells out into the lumen such as to occlude it or to form a papillary growth into it. No endothelial hyperplasia beyond that just mentioned has been seen.

The vessels are usually large cavernous spaces which throughout the series of cases are more or less constant in size. In the case reported by Guillain, Decourt, and Bertrand,[16] they were stated to be 3 to 4 mm. in diameter. However, in one instance, a hemangioma of the lower end of the ulna (Case 5), the vessels are much smaller, the lumina corresponding to those of capillaries (Fig. 3*b*). We have been unable to find another example of a capillary hemangioma involving bone. Nauwerck [17] called his case a capillary angioma although it was obviously composed of cavernous spaces. The same is true of the case reported by Ballance.

Microscopically the bony trabeculae within the hemangioma show two changes, degenerative and formative. The degenerative changes are manifest in two ways, both of which seem equally prevalent, necrosis and absorption. In almost any section of a hemangioma of bone can be seen spicules of bone definitely undergoing necrosis (Fig. 4*a*). They are granular, poorly stained and contain no cells or lacunae. Also there are to be seen many spicules with depressions along their surfaces in which can be seen large multinucleated osteoclasts destroying the bone (Fig. 4*b*).

The formative changes are also divided into two groups. The more common of these is evidenced by the many spicules of newly formed bone about whose surface can be seen a closely packed layer of osteoblasts (Fig. 5*a*). The less common is the transformation of fibrocartilage into bone. Strips of fibrocartilage are seen from the edges of which project spicules of newly formed bone. Over the surface of the vertebral arch in Case 1, between the bone and the

muscle is a layer of fibrocartilage. Toward the side of the muscle it gradually passes over into fibrous connective tissue to which the muscle is attached. On the other side the cartilage shows a gradual transition into osteoid tissue and then into bone (Fig. 5*b*). This would seem to explain the means by which the bone extends at the surface if one may generalize from the study of hemangioma of the vertebrae.

Various stages of these changes in the bone itself have been observed by other workers. Mauguière states that in the case of hemangioma of the clavicle which he studied incomplete calcification of osteoid masses was observed. Töpfer, in studying his cases of hemangioma of the vertebrae, saw absorption of bone by osteoclasts as well as the formation of bone by osteoblasts which formed a layer along the bony trabeculae, just as we have observed in our cases. However, no one has noted the generation of bone by the ossification of strands of fibrocartilage unless Mauguière's statement can be so interpreted. Birch-Hirschfeld [18] saw a large amount of cartilaginous tissue in the case he reported but noted no ossification of it. It is not surprising that Töpfer did not observe this process on the surface of the vertebral arch in any of his cases, as the hemangioma was limited to the vertebral body in almost all cases, involved the vertebral arch in only three instances and in none was the hemangioma large enough to produce symptoms. The bony changes in the case of hemangioma of the humerus observed by Ballance are unique. In his case, although the hemangioma was limited to the shaft of the bone, there was marked absorption of the cortex which was demonstrable radiologically as well as very evident in the gross and microscopic preparations. No similar examples are to be found in the literature.

There is nothing in our experience which throws any light upon the etiology of these lesions and as a result we do not feel capable of deciding whether these lesions are neoplasms or vascular malformations. Clinically they are certainly benign tumors and microscopically there is no evidence of malignancy. Mitotic figures are never seen and the stroma is quite acellular, loose and fibrous. Certainly we are dealing with a benign, slowly growing tumor mass that, as a result of its presence and growth, produces a characteristic reaction in the bone in which it arises or which it invades. As we have previously pointed out (Bucy and Capp) there is no reason to

conclude that the changes within the bone itself, both destructive and formative, are a primary part of the neoplastic process, while there is every reason to believe that the changes are only secondary reactive phenomena.

REFERENCES

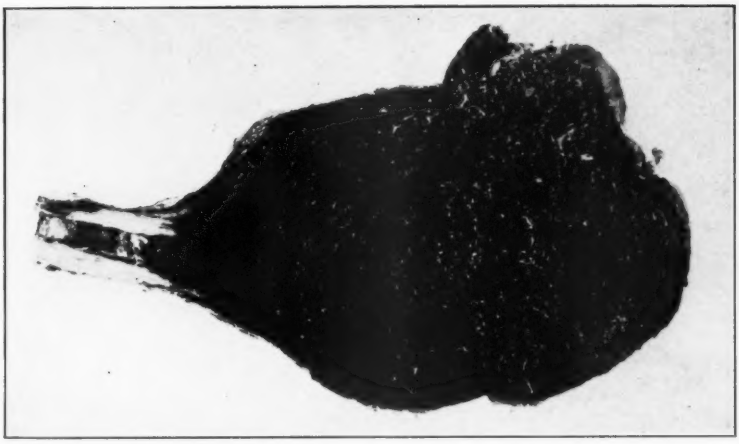
1. Bucy, P. C., and Capp, C. S. Primary hemangioma of bone. *Am. J. Roentgenol.* (in Press).
2. Töpfer, D. Über ein infiltrierend wachsendes Hämangiom der Haut und multiple Kapillarektasien der Haut und inneren Organe: zur Kenntnis der Wirbelangiome. *Frankfurt. Ztschr. f. Path.*, 1928, xxxvi, 337.
3. Stamm. Beitr. z. Lehre von Gefässgeschwülsten, Göttingen, 1891. Cited by Falkowski, *Beitr. z. path. Anat. u. z. allg. Pathol.*, 1914, lvii, 385.
4. Makrycostas, K. Ueber das Wirbelangiom, lipom und osteom. *Virchows Arch. f. path. Anat.*, 1927, cclxv, 259.
5. Cushing, H. Surgical end-results in general, with a case of cavernous hemangioma of the skull in particular. *Surg. Gynec. Obst.*, 1923, xxxvi, 303.
6. Codman, E. A. Bone Sarcoma. New York, 1925, 93.
7. Phemister, D. B. Bone tumors. *Northwest Med.* 1929, xxviii, 1.
8. Brandt, R. Zur Frage der Angiomatosis retinae. *Von Graefe's Arch. f. Ophth.*, 1921, cvi, 127.
9. Hitzrot, J. M. Hemangioma cavernosum of bone. *Ann. Surg.*, 1917, lxv, 476.
10. Mauguière, L. Angiosarcome de la clavicule. *J. de radiol. et d'électrol.*, 1920, iv, 269.
11. Zajaczkowski, A. Ein Fall von Angioma cavernosum des Stirnbeines. *Przeglad Chirurgiczny*, iv, no. 3. Abstr. *Centralbl. f. Chir.*, 1901, xxviii, 507.
12. Cushing, H., and Bailey, P. Hemangiomas of cerebellum and retina. *Arch. Ophth.*, 1928, lvii, 447.
13. Lücke, A. Ein Fall von Angioma ossificans der Highmorshöhle. *Deutsche Ztschr. f. Chir.*, 1890, xxx, 85.
14. Ballance, H. Intramedullary capillary angioma of shaft of humerus. *Brit. J. Surg.*, 1923–1924, xi, 622.
15. Perman, E. On haemangiomata in spinal column. *Acta. chir. Scandinav.*, 1926, lxi, 91.
16. Guillain, G., Decourt, J., and Bertrand, I. Compression médullaire par angiome vertébral. *Ann. de Méd.*, 1928, xxiii, 5.
17. Nauwerck, G. Über einen Fall von Zentralem hyperplastischem Kapillarangiom des Oberschenkels. *Virchows Arch. f. path. Anat.*, 1888, cxi, 211.
18. Birch-Hirschfeld, F. V. Lehrbuch der Pathologischen Anatomie. 1887. Cited by Schöne.
19. Schöne, G. Ueber einen Fall von Myelogenem Hämangiom des Os occipitale. *Beitr. z. path. Anat. u. z. allg. Pathol.*, 1905, vii, 685.

DESCRIPTION OF PLATES

PLATE 79

FIG. 1. Case 7. Hemangioma of clavicle on cut surface. (The saw has broken the cortex in the upper right corner.) Note the complete destruction of the cortex of the bone and the expanded but intact periosteal covering.

FIG. 2. (*a*) Case 2. Hemangioma of scapula. Hematoxylin and eosin. × 70.
(*b*) Case 1. Hemangioma of vertebrae. Note the small amount of intervascular connective tissue, its loose relatively acellular character. The single layer of flat endothelial cells is evident. Hematoxylin and eosin. × 85.



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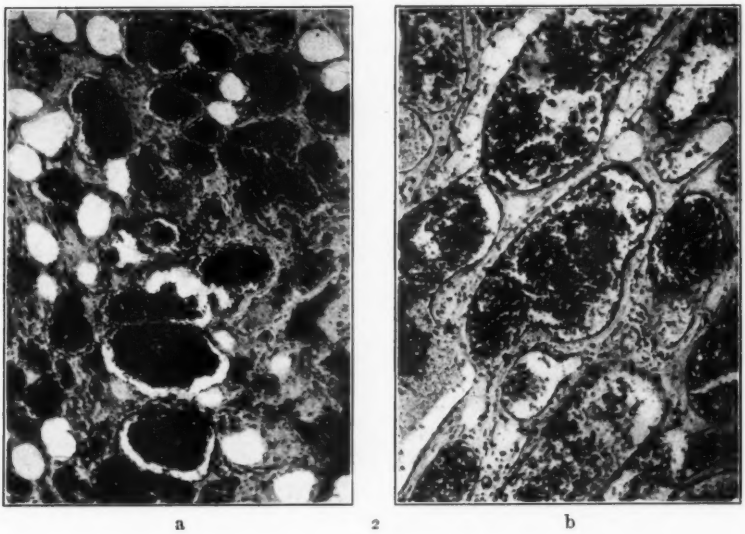


a 2 b

Bucy Hemangioma of Bone

PLATE 80

FIG. 3. (*a*) Case 3. Hemangioma of ilium. Large amount of connective tissue between the vascular sinuses. Hematoxylin and eosin. × 85.

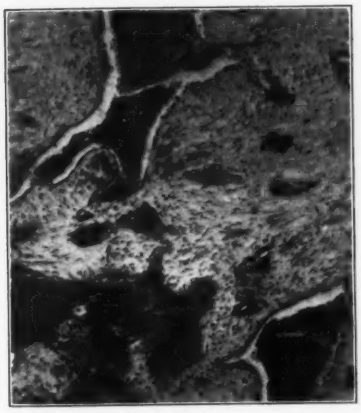
(*b*) Case 5. Hemangioma of ulna. Small capillary spaces with thick endothelial lining and a small amount of supporting connective tissue. Hematoxylin and eosin. × 85.

FIG. 4. (*a*) Case 3. A necrotic spicule of bone. × 85.

(*b*) Case 1. A multinucleated giant osteoclast resting in a depression in a bony spicule. × 275.

FIG. 5. (*a*) Case 7. Spicule of bone surrounded by a layer of osteoblasts. Hematoxylin and eosin. × 85.

(*b*) Case 1. Surface of the vertebral arch showing normal bone with underlying normal marrow below and above it a gradual transition through bone, osteoid tissue, fibrocartilage, and finally fibrous connective tissue. The latter is not shown here. × 85.



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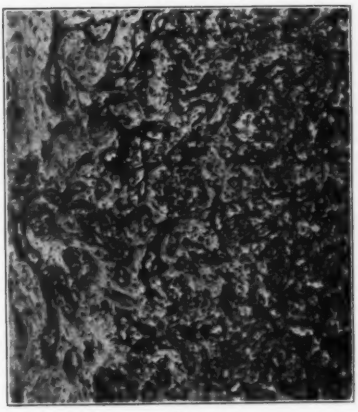

b

a

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b

a

5

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Bucy

Hemangioma of Bone

CONGENITAL MALFORMATION OF HEART WITH ANOMALOUS INSERTION OF PULMONARY VEINS, ABSENCE OF SPLEEN, SITUS INVERSUS OF ABDOMINAL VISCERA AND OTHER DEVELOPMENTAL ERRORS *

Report of a Case

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It is generally recognized that congenital malformation of the heart is frequently associated with abnormal development of its great vessels and occasionally with anomalies in other organs. In the following case the combination of cardiac malformation and other abnormalities is so unusual that a report of the case seems warranted.

Case Report

Clinical History: Baby Y, male, 7 months old, was suddenly seized with convulsions on Nov. 14, 1925, and died on the way to the Peking Union Medical College Hospital. There was nothing important in his past history except that whenever he cried his lips turned blue.

Necropsy

Gross Examination: This shows a well developed and nourished male Chinese infant, weighing 7,850 gm. The anterior fontanelle is open, 3 by 4 cm. in size, and the posterior fontanelle is closed. Eyes, ears, nose and mouth are normal. No teeth are present. Chest and abdomen are normal. There is marked clubbing of finger and toe nails.

Peritoneal Cavity: No free fluid nor adhesions are present in the peritoneal cavity. The surfaces are smooth and shining. The positions of the abdominal organs correspond to the mirror image of the normal. The liver edge is 5 cm. below the left costal margin and 4 cm. below the right costal margin. The gall bladder is hidden under the liver, its tip just reaching the lower edge of the liver in the

* Received for publication May 28, 1929.

midline. The round ligament is in the left upper quadrant. The stomach, moderately contracted, is found under the liver in the right upper quadrant. The ascending colon is on the left side of the abdominal cavity and the descending colon on the right, both attached to the mesocolon instead of to the abdominal wall. The appendix, 6 cm. long is shifted to the middle of the lower abdomen. The tail of the pancreas points to the right. No spleen is found. The mesenteric lymph nodes are enlarged and congested. The diaphragm reaches the level of the 5th intercostal space on each side. The inferior vena cava is on the left side of the vertebral column and the abdominal aorta on the right.

Pleural and Pericardial Cavities: Normal.

Heart: The heart is distinctly larger than normal. Viewed anteriorly when the organ lies *in situ* (Fig. 1) the surface is smooth, showing no evidence of interventricular groove. The apex is very broad and rounded. Between the auricular appendages at the base lies the ascending portion of the aortic arch. The pulmonary artery is not seen. On lifting up the apex and throwing the heart upward and backward (Fig. 2) two superior venae cavae are seen, one right and one left, each emptying into its respective auricle. The inferior vena cava opens into the postero-inferior aspect of the right auricle. The pulmonary artery is smaller than usual and has its origin posterior to that of the aorta (Fig. 6). None of the pulmonary veins is found to enter the heart directly. On the posterior surface of the large ventricle there is a small crescent-shaped area surrounded by a zone of fat tissue, suggesting the external surface of a rudimentary right ventricle which, however, is not present. The right auricle is much dilated (Fig. 3). The interauricular septum which forms the left wall of this auricle, is incomplete. It is represented by a small band of tissue bridging the auricle anteroposteriorly and almost horizontally. It is attached to the anterior auricular wall at the auriculoventricular junction and to the posterior auricular wall midway between the openings of the right superior vena cava and the inferior vena cava (Figs. 3, 4 and 5). The right auricle has five openings: (1 and 2) the two interauricular foramina, (3) the opening of the right superior vena cava, (4) the opening of the inferior vena cava, and (5) the auriculoventricular opening. Each of the two interauricular foramina, which are one above the other, is about 1.5 cm. in diameter. The upper one is above the band of the de-

fective interauricular septum and the other below. The opening of the right superior vena cava is on the right side of the upper foramen, looking into both the right and the left auricles. The opening of the inferior vena cava is on the right side of the lower foramen, also looking into both the right and the left auricles. The auriculoventricular opening is guarded by a valve consisting of two cusps not well formed but probably competent. The left auricle is smaller than the right. At its upper border is the opening of the left superior vena cava, and in its posterior wall is that of the coronary sinus (Fig. 5). There is no direct communication between the left auricle and the ventricle. The blood in the left auricle must pass through the interauricular foramina into the right auricle before reaching the ventricle. The ventricle consists of a single chamber narrow anteroposteriorly but wide laterally. There are several large papillary muscles extending from the broad apex upward and backward. This ventricle has three openings: (1) the auriculoventricular opening already described, (2) the opening of the aorta, and (3) the opening of the pulmonary artery. The aorta arises from the left ventricular base and has three normal cusps (Fig. 5). The pulmonary artery originates directly behind the aortic opening. It is much smaller than normal and has a very thin wall like that of a vein. The pulmonary valve is a small funnel-shaped structure with its convex surface on the arterial side. In its middle is the pulmonary opening the size of a pin-point (Fig. 7). The infundibulum is narrow but its musculature is hyperplastic.

Arteries: There are two coronary arteries, the anterior and the posterior. The anterior coronary artery (corresponding to the normal left coronary artery) arises from the aortic wall on the left side, just behind the left aortic cusp (Fig. 5), and passes downward to the anterior surface of the ventricle (Fig. 1). The larger posterior coronary artery (corresponding to the normal right coronary artery) arises from the posterior part of the aortic wall behind the posterior aortic cusp (Figs. 5 and 6). It passes downward for a few millimeters and then gives off three branches (Fig. 6): (1) the left branch, extending to the left along the auriculoventricular groove and downward toward the apex on the left side of the posterior surface, (2) the auricular branch, and (3) the right branch which forms a semicircle around the right half of the auriculoventricular groove. From its proximal portion, it gives off two branches each of which passes

forward to the anterior surface of the heart (Fig. 1). The ductus arteriosus is not found. The pulmonary artery divides into left and right branches which pass directly into the lungs (Fig. 2). From the aortic arch there arise three branches in the order given: (1) the innominate artery which is unusually short, (2) the left common carotid artery and (3) the left subclavian artery (Fig. 1). Between the innominate and the common carotid are two very small twigs, one in front of the other. They are not over ½ mm. in diameter and pass straight upward (Fig. 1). They are probably the thyroidea ima arteries. The descending aorta is found on the right side of the spinal column. The celiac axis, the gastric, splenic and left gastro-epiploic arteries are absent. The superior mesenteric artery arises just a little below the level of the renal arteries (Fig. 9). It passes downward behind the duodenum, where it divides into two branches, the gastrohepatic and the duodenal. The gastric branch runs along the greater curvature of the stomach and sends a few small twigs into the pancreas. The hepatic branch bifurcates shortly before it enters the liver. The left branch, just before entering the liver, sends off a small twig into the wall of the gall bladder. The duodenal artery is much shorter and smaller than the gastrohepatic and supplies only the duodenum. The proximal half of each hypogastric artery is patent.

Veins: There are two transverse pulmonary veins, one above the other (Fig. 2). The upper one which receives blood from all the lobes of both lungs, has no external connection whatever but is connected with the lower transverse vessel through anastomosing vessels in the lung tissue. The lower transverse vessel joins with the large gastric vein along the left side of the lower part of the esophagus and the lesser curvature of the stomach. This gastric vein joins in turn with the portal vein just before the latter enters the liver (Figs. 2 and 10). Thus the blood from the pulmonary vein must pass through the liver before it reaches the heart again. The left descending and ascending azygos veins join together at the level of the fifth thoracic vertebra and empty into the left superior vena cava (Figs. 1 and 2). The right ascending azygos vein passes upward and joins the left azygos before the latter enters the left superior vena cava. The connection between the five or six right upper intercostal veins and the right superior vena cava was unfortunately destroyed at necropsy. The left and the right mammary veins empty into their respective

superior venae cavae. The inferior vena cava is found on the left side of the spinal column but as it passes through the fissure on the left of the Spigelian lobe of the liver it takes a sharp turn toward the right and receives blood from the three hepatic veins (Fig. 10). It then passes through the opening in the diaphragm and enters into the right auricle at its base. The right renal vein crosses the aorta on its anterior surface (Fig. 8). The right spermatic vein empties into the right renal vein.

Lungs: Each lung has three lobes.

Liver: The size of the liver is normal and its shape rectangular (Fig. 12). The left lobe is thick with rounded edges, while the right lobe is thin and can be rolled upon itself. There is no caudate lobe. The tip of the Spigelian lobe points upward and backward. The quadrate lobe is represented by a slight swelling on the lower part of the posterior surface of the left lobe. The fissure for the inferior vena cava is on the left of the Spigelian lobe and the fissure of the ductus venosus is on the right. Below the transverse fissure is the wide, shallow fissure for the gall bladder which is on the right of the swelling of the quadrate lobe. No umbilical fissure is found externally, but when the swelling of the quadrate lobe is cut through, a canal is exposed containing the round ligament.

Gall Bladder and Bile Ducts: They are normal.

Pancreas: The position of the pancreas is reversed, with its head pointing to the left and its tail to the right. The pancreatic duct opens separately into the duodenum at a point about 1.5 cm. above the ampulla of Vater (Fig. 11).

Gastro-Intestinal Tract: The esophagus is normal. Its position is slightly shifted to the right; but its relation to the trachea is not changed. The stomach is transposed to the right. The small and large intestines show very prominent Peyer's patches and lymph follicles.

Adrenal Glands: The left adrenal gland is triangular and is attached to the inferior surface of liver. The right is crescent-shaped.

Diaphragm: The aortic opening in the diaphragm has shifted slightly to the right. The esophageal opening is directly in front of the aortic opening. The opening for the inferior vena cava is on the left of the esophageal opening.

Thymus: The thymus weighs 24 gm. and measures 5.5 by 5.5 by 1.8 cm.

Lymph Nodes: The cervical and other peripheral lymph nodes are normal in appearance. The mesenteric and the retroperitoneal nodes are markedly enlarged.

Other Organs and Tissues: Kidneys, testes, brain, pituitary gland and bone marrow of femur are normal.

Histology: Microscopic examination shows no change of importance, except for the hyperplasia of the lymphoid tissue in thymus, tonsils, and the small and large intestines.

Summary of Findings

Congenital malformation of the heart consisting of one imperfectly separated auricle and one common ventricle; patent septum primum and secundum; transposition of pulmonary artery and aorta; hypoplasia of pulmonary artery; persistent left superior vena cava, non-development of innominate vein; anomalous drainage of the pulmonary veins into the portal circulation; anomalous drainage of the azygos veins into the left superior vena cava; situs inversus of all abdominal organs; absence of celiac axis and anomalous origin of the gastrohepatic and duodenal arteries from the superior mesenteric; patent hypogastric arteries; malformation of the left lung; absence of spleen; hyperplasia of mesenteric and retroperitoneal lymph nodes; persistence of ascending and descending mesocolon; anomalous insertion of the pancreatic duct into the duodenum.

Comment

There are several features in this case on which brief comment may be made. The malformation of the heart is very marked. Although functionally the heart is biloculate, morphologically it is an imperfect triloculate heart. The common auricle is incompletely divided into left and right by a narrow band of tissue representing the septum primum the development of which stopped at the stage when both the ostium primum (interauricular foramen) below and the ostium secundum (foramen ovale) above were present. The septum secundum has never developed. This imperfect formation of the interauricular septum may be secondary to the failure of the pulmonary vein to insert into the auricle. The persistence of the common auriculoventricular orifice is due to the arrested development of the endocardial cushions associated with the non-appearance

of the interventricular septum. The left superior vena cava represents the persistent left common cardinal vein. The normal anastomosis between the left and the right anterior cardinal veins, which later form the left innominate vein, has failed to develop. The pulmonary artery is abnormal both in the degree of its development and in its relation to the aorta. The rudimentary condition of the valve is ordinarily described as pulmonary atresia and is consequent upon the non-appearance of the interventricular septum, as a result of which most of the blood in the common ventricle empties into the aorta. The origin of the pulmonary artery from the posterior aspect of the aorta and its direct course to the lungs can be ascribed to the failure on the part of the endocardial folds of the bulbus cordis to form the usual spiral arrangement. This causes the bulbus to retain its early embryological straight form, resulting in the transposition of the aorta and the pulmonary artery. In this case the transposed vessels are connected with the common ventricle and, in the absence of the interventricular septum, it is difficult to determine whether this transposition might be "corrected" or "uncorrected." The anomalous drainage of the pulmonary veins into the portal system is one of the most interesting findings in this case. In order to understand this unusual phenomenon it is well to refer to the observations of Buell [1] on the development of pulmonary vessels in chick embryos. He finds that in the dorsal endothelial wall of the sinus venosus at the level of the developing lung bud in the chick embryo, there is a proliferation of angioblastic cells extending dorsad until the ventral surface of the gut is encountered. They then grow in all directions over the ventral surface of the gut, contributing to the formation of the splanchnic plexus. This plexus, when fully developed, can be divided roughly into two groups: (1) the group which persists, forms connection with the sinus venosus and later develops into pulmonary veins and (2) the group which forms the cranial and postcaval portions of the splanchnic plexus, and quickly disappears. Occasionally, however, some of the postcaval connections of the splanchnic plexus may persist, while the normal connection between the plexus and the sinus venosus may fail to develop. This results in an absence of connection between the heart and the pulmonary veins, and the formation of a new connection between these veins and the portal system as in the case here described. The absence of spleen has been found in other cases in

association with malformation of the heart and anomalous insertion of the pulmonary veins. Sometimes, instead of the spleen, other organs, such as the kidney, may be absent. The pancreatic opening into the duodenum represents that of the dorsal pancreatic duct (duct of Santorini), which in this case persists. The ventral duct and its opening in the ampulla of Vater are not found, suggesting that probably the ventral pancreas has never developed.

REFERENCE

1. Buell, C. E., Jr. Origin of the pulmonary vessels in the chick. *Contributions to Embryology, No. 66, Carnegie Institution of Washington*, xiv, 1922.

DESCRIPTION OF PLATES

Plate 81

Fig. 1. Diagram showing the heart and the relation of its big vessels. The lungs are retracted. Anterior view.

Fig. 2. Diagram showing the vessels of the lungs and the connection of the pulmonary veins with the gastric vein.

KEY TO THE PLATES

A ..... Aorta.
A. B. ..... Auricular branch of posterior coronary artery.
A. C. A. ... Anterior coronary artery.
A. C. A. O. . Opening of anterior coronary artery.
A. V. V. ... Auriculoventricular valve.
Az. V. ..... Azygos vein.
Amp. V..... Ampulla of Vater.
C. D. ..... Cystic duct.
Com. D. ... Common duct.
C. S. ..... Opening of coronary sinus.
Cys. A. .... Cystic artery.
D. A. ..... Duodenal artery.
Diaph. .... Diaphragm.
Duod. ..... Duodenum.
Eu. V. ..... Eustachian valve.
F. G. B. ... Fissure for gall bladder.
G. E. A. ... Gastro-epiploic artery.
G. E. V. ... Gastro-epiploic vein.
G. H. A. ... Gastrohepatic artery.
G. V. ..... Gastric vein.
H. A. ..... Hepatic artery.
H. V. ..... Hepatic veins.
I. M. A. ... Inferior mesenteric artery.
I. V. C. .... Inferior vena cava.
I. V. C. O. . Opening of inferior vena cava into right portion of the common auricle.
In. A. ..... Innominate artery.
L. A. ..... Left auricle.
L. Anas..... Anastomosis between pulmonary veins of the left lung.
L. B..... Left branch of posterior coronary artery.
L. C. C. A. . Left common carotid artery.
L. C. I. A. . Left common iliac artery.
L. Ex. I. A Left external iliac artery.
L. Hy. A.... Left hypogastric artery.
L. Int. I. A . Left internal iliac artery.
L. L. L. .... Lower lobe of left lung.
L. M. L. ... Middle lobe of left lung.
L. M. V. ... Left mammary vein.
L. P. A. .... Left pulmonary artery.
L. R. A. ... Left renal artery.
L. R. V. ... Left renal vein.
L. S. A. .... Left subclavian artery.
L. S. V. C. . Left superior vena cava.
L. U. L. ... Upper lobe of left lung.
Lo. P. V. .... Lower pulmonary vein.
Mes. ..... Mesentery.
O. P. D. ... Opening of pancreatic duct into duodenum.
Oesoph. ... Oesophagus.
P. A. ..... Pulmonary artery.
P. C. A. ... Posterior coronary artery.
P. C. A. L. . Lateral branch of the right branch of the posterior coronary artery.

(*Continued on following page*)

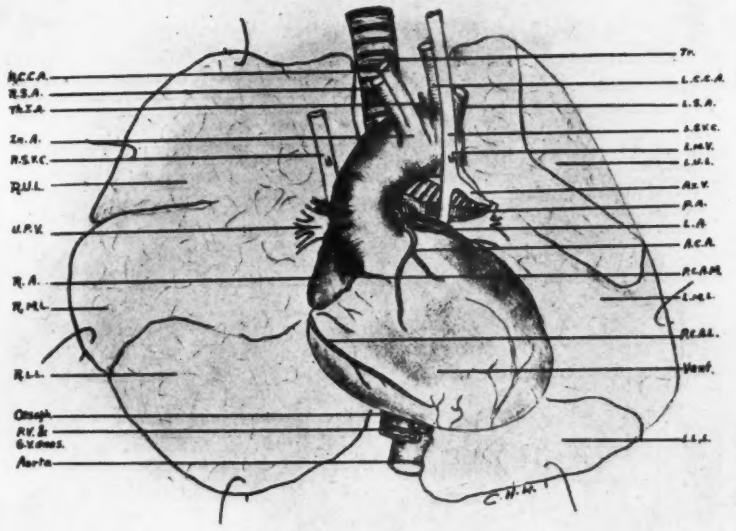
R.C.C.A.
R.S.A.
T.h.I.A.
In.A.
R.S.V.C.
R.U.L.
U.P.V.
R.A.
R.M.L.
R.L.L.
Oesoph.
R.V. & G.V. Anas.
Aorta
Tr.
L.C.C.A.
L.S.A.
L.S.V.C.
L.M.V.
L.M.L.
Az.V.
P.A.
L.A.
A.C.A.
R.C.A.M.
L.M.L.
R.C.L.L.
Vent.
L.L.L.
C.H.W.

Fig 1

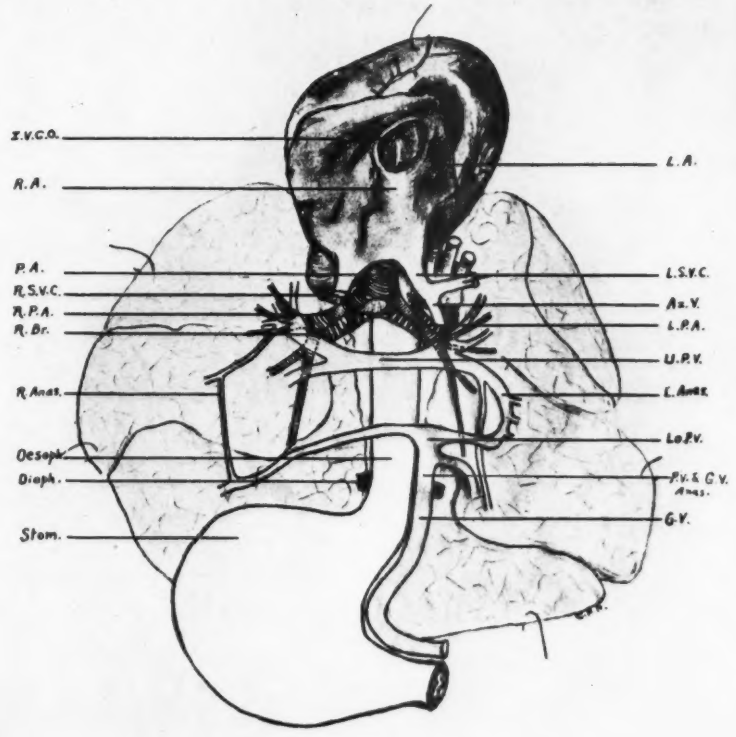
I.V.C.O.
R.A.
P.A.
R.S.V.C.
R.P.A.
R. Br.
R. Anat.
Oesoph.
Diaph.
Stom.
L.A.
L.S.V.C.
Az.V.
L.P.A.
U.P.V.
L. Anat.
Lo.P.V.
P.V. & G.V. Anas.
G.V.

Fig. 2

PLATE 82

FIG. 3. Posterior and inferior views of the heart with the right auricle cut open. Bristle 1 passes through the auriculoventricular opening and out through a window in the right side of the common ventricle. Bristle 2 passes through the right superior vena cava to the right auricle. Bristles 3 and 4 pass through the left superior vena cava to the left auricle. The lower end of Bristle 3 is seen through the foramen secundum (foramen ovale) and that of 4 is seen through foramen primum (interauricular foramen). Bristle 5 passes through the foramen secundum (foramen ovale) into the left auricle and then through the foramen primum (interauricular foramen) out again to the right auricle.

FIG. 4. Sagittal section of the heart through the right auricle and the common auriculoventricular opening. Bristle 2 passes through the right superior vena cava to the right auricle. Bristle 4 passes through the left superior vena cava to the left auricle. Bristle 6 passes through the opening of inferior vena cava into right auricle and then through the common auriculoventricular opening into the common ventricle. Bristle 7 passes through the aorta into the left portion of the common ventricle.

FIG. 5. Sagittal section of the heart through the left auricle, aortic opening and the left portion of the common ventricle. Bristle 2 passes through right superior vena cava into right auricle. Its lower end seen through the auricular foramen. Bristle 3 passes through left superior vena cava into left auricle. Bristle 8 passes through opening of inferior vena cava into right auricle. Bristle 9 passes through the common auriculoventricular opening.

FIG. 6. Posterior and inferior views of the heart with the auricular appendages and the superior venae cavae retracted in order to show the origin of the pulmonary artery and the origin and distribution of the posterior coronary artery.

KEY TO THE PLATES (*Continued*)

P. C. A. M. Medial branch of the right branch of the posterior coronary artery.
P. V. & G. V. Anas. Anastomosis between pulmonary and gastric veins.
P. Va. ..... Pulmonary valve.
Panc. ..... Pancreas.
Panc. A. ... Pancreatic artery.
Panc. V. ... Pancreatic vein.
Phr. A ..... Phrenic artery
Por V. .... Portal vein.
R. A. ..... Right auricle.
R. Anas. ... Anastomosis between the pulmonary veins of the right lung.
R. B. ..... Right branch of posterior coronary artery.
R. Br. ..... Right bronchus.
R. C. C. A. . Right common carotid artery.
R. C. I. A. . Right common iliac artery.
R. Ex. I. A. Right external iliac artery.
R. Hy. A. .. Right hypogastric artery.
R. Int. I. A. Right internal iliac artery.
R. L. L. ... Lower lobe of right lung.
R. M. L. ... Middle lobe of right lung.
R. P. A. ... Right pulmonary artery.
R. R. A..... Right renal artery.
R. R. V..... Right renal vein.
R. S. A. ... Right subclavian artery.
R. S. V. C. . Right superior vena cava.
R. Sp. V. .. Right spermatic vein.
R. Sup. A. . Right suprarenal artery.
R. U. L. ... Upper lobe of right lung.
S. M. A..... Superior mesenteric artery.
S. M. V..... Superior mesenteric vein.
Stom. ..... Stomach.
Th. I. A. ... Thyroidea ima arteries (?).
Tr. ..... Trachea.
U. P. V. ... Upper pulmonary vein.
Vent. ..... Common ventricle.

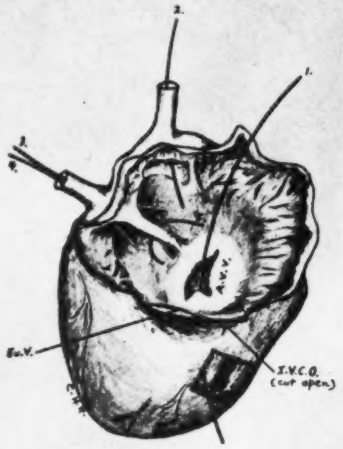

Fig. 3

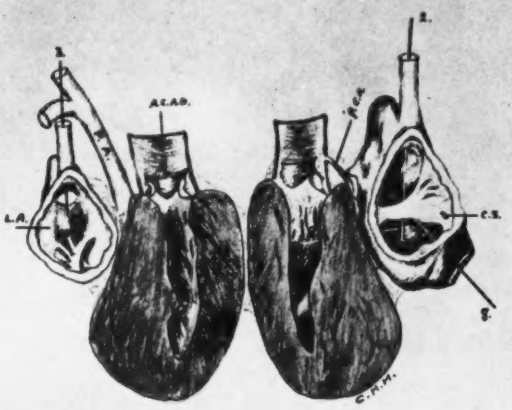

Fig. 5

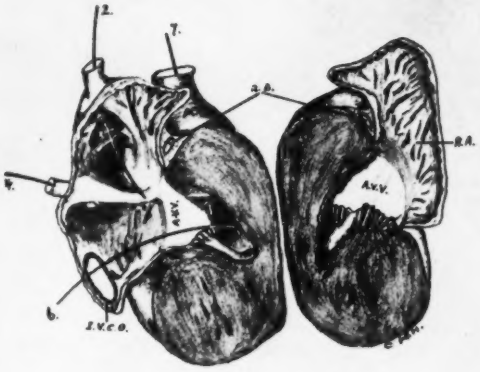

Fig. 4

Fig. 6

Plate 83

Fig. 7. Pulmonary artery and valve, cut open.

Fig. 8. Diagram showing the relations of the renal vessels, anterior view. x (?) Right suprarenal vein. xx (?) left spermatic vein.

Fig. 9. Diagram showing the arteries of stomach, intestine, pancreas, liver and gall bladder.

Fig. 10. Diagram showing the portal vein and its tributaries.

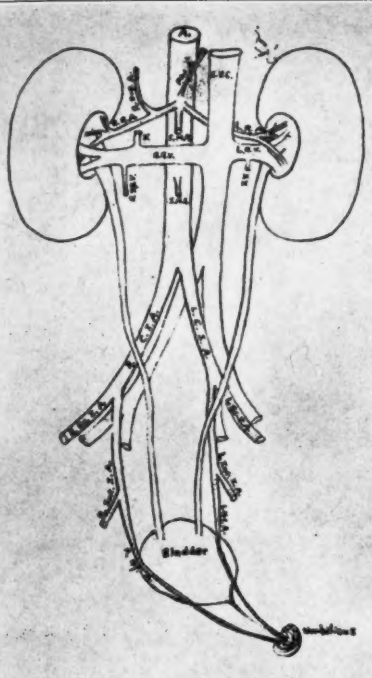

Fig. 8

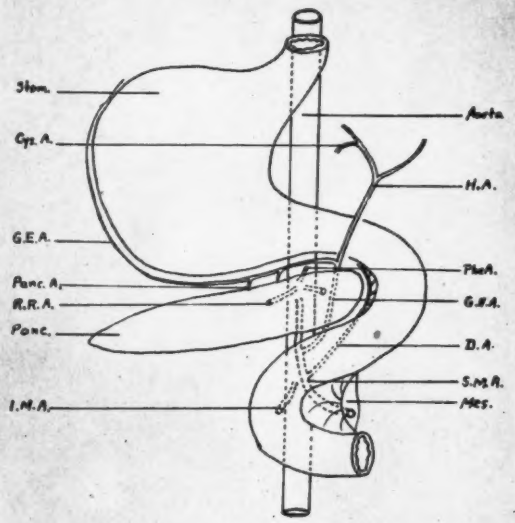

Fig. 9

Fig. 7

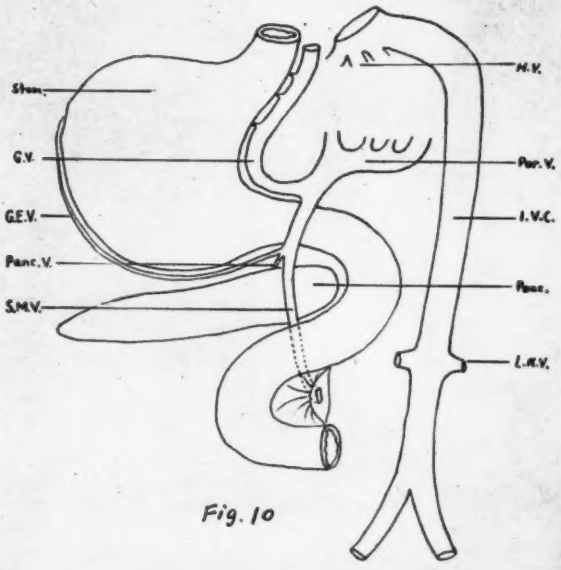

Fig. 10

PLATE 84

FIG. 11. Diagram showing the relation between the openings of the bile and pancreatic ducts.

FIG. 12. Posterio-inferior view of the liver, showing the fissures of the organ. Arrow indicates the direction in which the inferior vena cava passes into the heart.

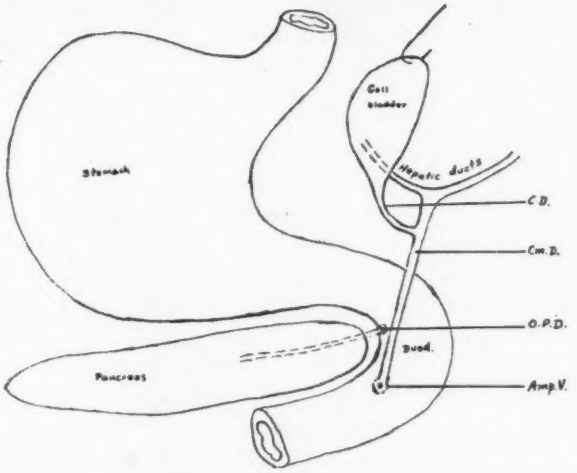
Gall bladder
Stomach
Hepatic ducts
C.D.
Cm.D.
O.P.D.
Duod.
Pancreas
Amp.V.

Fig. 11

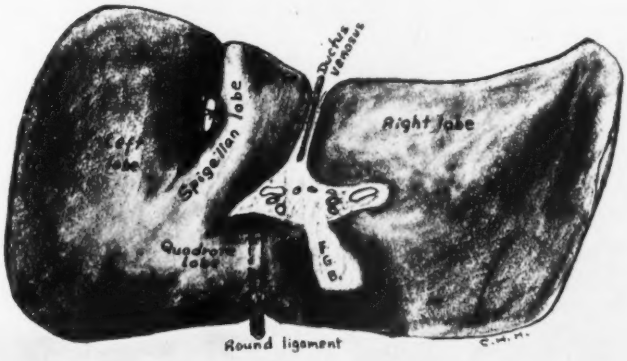
Ductus Venosus
Left lobe
Spigelian lobe
Right lobe
Quadrate lobe
G.B.
Round ligament

Fig. 12

INVOLVEMENT OF MEDIUM-SIZED ARTERIES ASSOCIATED WITH SYPHILITIC AORTITIS *

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Much has been written about syphilis of the aorta and about syphilis of smaller arteries, especially arteries of the brain, but syphilis of medium-sized arteries has attracted little attention. In a previous communication [1] the extension of syphilis to the aortic valves was described. The purpose of this paper is to describe the manifestation of the syphilitic process in the other direction, namely in the branches of the aorta. The syphilitic lesions in the aorta seen at autopsy are as a rule old, and hardly any conclusions as to the pathogenesis of the process can be drawn from the old cases. The present study was undertaken as a search for initial or early stages of syphilis in types of vessels whose structures closely resemble that of the aorta. The term medium-sized arteries is used for convenience but it has the sanction of authorities on anatomical nomenclature.

LITERATURE

In 1922 Warthin [2] reported syphilitic lesions in branches of the aorta. His investigation was confined mainly to branches of the descending aorta. He described changes in the carotid in only one case, and changes in the subclavian artery in several cases. In his opinion these changes are essentially the same as are found in syphilitic aortitis. As to the relation of syphilis and arteriosclerosis, he further stated that simple arteriosclerosis of smaller and medium-sized vessels is more common in syphilitics than in non-syphilitics. Warthin, however, did not mention the number of cases examined, nor did he give any details referring to the frequency of the finding of syphilitic changes in the various vessels. MacKenzie [3] stated, (quoting Turnbull) that among two hundred and eighty-eight cases of syphilis, specific lesions were found twice in the coeliac artery and once in the superior mesenteric and splenic artery. Lange,[4] in his

* Received for publication May 17, 1929.

chapter on syphilis of the arteries, stated that the changes in the medium-sized arteries in syphilis are similar to those found in the smaller arteries but described changes of the arteries of this latter type only. Letulle [5] and his co-workers published a case of intermittent claudication which at autopsy showed syphilitic changes of the splenic, common and external iliac, and both tibial arteries. The textbooks of pathology with the exception of MacCallum [6] give detailed descriptions of syphilitic changes of the aorta and smaller arteries, but do not refer to the involvement of the medium-sized arteries.

Material and Methods

Fifty cases which at autopsy showed grossly and histologically syphilitic lesions in the aorta were used for this investigation. Blocks of portions of the innominate, common carotid, superior and inferior mesenteric, common iliac and femoral arteries were taken from these fifty cases and, in the majority of them, from the subclavian also. Owing to the fact that the autopsies were limited to thorax and abdomen, and that syphilitic changes of the vessels in question were more likely to be encountered close to the aorta, the blocks were taken exclusively from these portions. The sections were stained with hematoxylin-eosin, orcein, and a combination of iron hematoxylin and orcein. This combined stain was found especially useful in staining both elastic and nuclear elements in one section. The method of combination of the orcein and iron hematoxylin stain is as follows:

1. Paraffin sections, xylol, alcohol, water.
2. Alcoholic solution of orcein, for one hour in incubator at 37° C. (Orcein, Grübler 0.5 gm., absolute alcohol 40 cc., distilled water 20 cc., hydrochloric acid, C. P., 20 drops.)
3. Water.
4. Acid alcohol, for six seconds. (Alcohol 95 per cent, 200 cc., distilled water 50 cc., hydrochloric acid, C. P., 1 cc.)
5. Water.
6. Iron hematoxylin, 40 minutes. (Solution *a*: Hematoxylin 10 gm., alcohol 95 per cent, 100 cc. Solution *b*: Liquor ferri sesquichlorate 4 cc., distilled water 95 cc., hydrochloric acid, C. P., 1 cc. Equal parts of *a* and *b* are mixed and filtered each time before using.)
7. Acid alcohol. (1 part 2 per cent acid alcohol, 2 parts of water.)
8. Ammonium alcohol, for a few seconds. (30 per cent alcohol with a few drops of ammonium hydroxide 28 per cent.)
9. Water. (Over night.)

10. 95 per cent alcohol to which a few crystals of picric acid are added. 2 to 2½ minutes.
11. Alcohol 95 per cent, absolute, xylol.

Connective tissue and muscle fibers were stained according to Van Gieson's method, and Warthin–Starry's and Levaditi's methods were used in the search for spirochetes.

Results

In a previous paper it has been shown that the earliest manifestation of syphilis of the aorta is in the adventitia in the form of endarteritis obliterans of the vasa vasorum and perivascular infiltration of lymphocytes. These two factors were used as criteria for the presence of early syphilis in medium-sized arteries. Interruption of the elastic lamellae of the media by round cell infiltration or connective tissue in these areas was interpreted as a later manifestation of syphilis.

Innominate Artery: In thirty-three of the fifty cases the innominate artery showed definite signs of syphilis. Some of the vasa vasorum of the adventitia showed a distinct intimal proliferation, resulting sometimes in an almost complete obliteration of the lumina. In some sections there was a varying amount of perivascular infiltration of lymphocytes and plasma cells. In some of the cases a new formation of connective tissue was found in the adventitia. The more pronounced the lymphocytic infiltration, the more connective tissue was observed in the layer. Wherever lymphnodes were found in the adventitia, they seemed hyperplastic. The media in twenty-three cases disclosed an interruption of the elastic lamellae. Some of the vessels presented perivascular infiltration of lymphocytes in the media, but plasma cells were also found in these regions. Older cases showed a new formation of connective tissue throughout the media. This connective tissue was very poor in nuclei and contained small portions of broken up elastic fibers. However, in ten cases the media was free from pathological changes. Some of the sections showed a marked proliferation of the intima with hyalinization and calcification; other sections showed atheromatous cavities, the inner surface of some of which was broken down, leading to atheromatous ulcers covered with thrombi. Some of the sections showed a slight circumscribed proliferation of connective tissue of the subendothelial layer. Throughout this connective tissue a slight infiltration of

lymphocytes was observed. No fatty changes could be made out in these portions.

Carotid Artery: The changes were essentially the same as were encountered in the innominate artery. In twenty-nine cases syphilitic changes were seen in the carotid artery. Whereas in fourteen of these cases the adventitia showed perivascular infiltration of lymphocytes, the media was apparently free from pathological changes. The changes of the intima, especially the circumscribed proliferations of connective tissue, could be studied to a greater advantage in the carotid artery because of the less severe concomitant arteriosclerosis. In serial sections which were cut through the wall of the artery at the site of the circumscribed proliferation of connective tissue of the intima, it was demonstrated that the corresponding portion of the adventitia showed a circumscribed perivascular infiltration of lymphocytes and plasma cells. The adventitia in other portions in some of these vessels was normal. Only the portion of the intima just above these areas offered the circumscribed button-like proliferation of connective tissue. The connective tissue showed a few spindle cells and a slight infiltration of lymphocytes and plasma cells. Between the inner portions of the media and this newly formed connective tissue, a few small-sized blood vessels were seen. There was no multiplication of the lining endothelial cells of these vessels. Degenerative changes were not observed.

Subclavian Artery: The subclavian artery was examined in twenty-nine cases, and in fifteen showed syphilitic lesions. The adventitia alone was the seat of perivascular infiltration of lymphocytes in seven cases. The intima in a majority of cases showed a similar circumscribed proliferation of connective tissue at the site of the perivascular infiltration of lymphocytes in the adventitia. In one case, circumscribed areas were found in the media consisting of endothelial cells, lymphocytes, plasma cells and several giant cells, combined with some foci of necrosis. These areas can be interpreted as gummata. In every other respect the changes were essentially the same as were found in the innominate artery.

Common Iliac Artery: The common iliac artery was involved in ten cases. The changes in the adventitia, media and intima correspond to the findings in the arteries discussed above. In addition to the circumscribed proliferation of connective tissue in the intima, large areas of arteriosclerosis were observed throughout the sections.

Whereas the arteries so far described have an excess of elastic tissue, the arteries which will be discussed subsequently show an excess of the muscular tissue.

Superior Mesenteric Artery: Changes suggestive for syphilis were encountered in the superior mesenteric artery in ten cases. The adventitia showed a varying amount of perivascular infiltration of lymphocytes and plasma cells. A proliferation of the lining endothelial cells of the vasa vasorum was much less often observed than in the arteries discussed above. A few slides showed a rather diffuse infiltration of a few lymphocytes, plasma cells and endothelial cells. The media was much less frequently involved than the adventitia, as changes were found only in one case. Here the changes were present mainly in the outer portions of the media corresponding to the places where most of the elastic fibers were seen, while the inner, more muscular parts showed no changes. The outer part of the media showed an interruption of the elastic lamellae, circumscribed areas of lymphocytic infiltration and some fibrosis. The intima showed in most of these cases a marked, well circumscribed new formation of connective tissue at the site where the perivascular infiltrations of lymphocytes were found in the adventitia. This circumscribed newly formed connective tissue bulged into the lumen, resulting in a narrowing and alteration of the shape of the lumen. There was a splitting of the internal elastic lamellae in several places. Fatty degeneration in the intima or other retrogressive changes were not noted in these circumscribed areas, but were extreme in slides showing diffuse intimal proliferation.

Inferior Mesenteric Artery: Changes characteristic of syphilis were observed in three cases. Here too, mainly perivascular infiltrations of lymphocytes and plasma cells were found in the adventitia, but only very few vasa vasorum showed endarteritis. The media was practically free from pathological lesions, while the intima showed changes similar to those found in the superior mesenteric artery.

Femoral Artery: In only seven cases did the femoral artery show evidence of syphilis, as indicated by a perivascular infiltration of lymphocytes and a few plasma cells. The vasa vasorum showed a moderate degree of proliferation of the lining endothelial cells. There was some fibrosis of the adventitia. The media, in its outer third, showed circumscribed lymphocytic infiltration. In only one

TABLE I

No.	Age	In.	Car.	*Subc.	Il.	Fem.	S.M.	I.M.
1	44	+	−		−	−	−	−
2	48	−	−		−	−	−	−
3	n.r.	−	−	−	−	−	−	−
4	35	+	−		−	+	−	−
5	25	+	+	−	−	−	−	−
6	53	+	+	+	+	−	−	−
7	78	−	−		−	−	−	−
8	45	−	+	−	−	−	−	−
9	59	+	+	+	+	−	−	−
10	70	+	−		−	+	−	−
11	62	+	+	+	−	−	−	−
12	60	−	−		−	−	−	−
13	35	+	+	+	−	+	+	+
14	36	+	+	+	−	−	−	−
15	45	+	+	−	−	−	−	−
16	51	−	−	−	−	−	−	−
17	43	−	−	−	−	−	−	−
18	25	−	−	+	−	−	+	+
19	52	−	−		−	−	−	−
20	48	+	+	+	+	−	+	−
21	50	−	+		−	+	−	−
22	66	−	−	−	−	−	−	−
23	50	+	+		−	+	−	−
24	52	+	+	−	−	−	−	−
25	30	+	+	+	−	−	+	−
26	49	+	+		+	−	+	−
27	67	+	+		+	−	−	−
28	50	−	−	−	−	−	−	−
29	41	+	+		+	−	−	−
30	49	−	−		−	−	−	−
31	61	−	−	−	−	−	−	−
32	52	−	−	−	−	−	−	−
33	46	−	−		+	−	−	−
34	51	+	+		+	−	−	−
35	65	+	+	+	−	−	−	−
36	49	+	+	+	−	−	−	−
37	58	+	−		−	−	−	−
38	45	+	+		−	−	+	−
39	31	+	+	+	−	−	+	+
40	60	+	+	+	+	−	+	−
41	43	+	+		−	−	−	−
42	34	+	+		−	−	−	−
43	75	+	−	−	−	−	−	−
44	35	+	+		−	−	−	−
45	60	+	+	+	−	−	−	−
46	52	+	+		−	+	+	−
47	40	−	−	−	−	−	−	−
48	54	+	−	−	−	−	−	−
49	65	+	+	+	−	−	−	−
50	46	+	+	+	+	+	+	−

\+ indicates syphilis is present.
− indicates absence of syphilis.
n.r. = no reference.
In. = innominate artery.
Car. = carotid artery.
Subc. = subclavian artery.
Il. = common iliac artery.
Fem. = femoral artery.
S.M. = superior mesenteric artery.
I.M. = inferior mesenteric artery.

* The subclavian artery was examined in twenty-nine cases.

case circular calcified plaques were found in the media, characteristic of Moenkeberg's arteriosclerosis. The intima showed both diffuse areas of arteriosclerosis and circumscribed fibrotic portions.

Table I summarizes the incidence of the involvement of the medium-sized arteries in syphilis. It also gives the ages of the patients.

Discussion

Most of the changes in this series of cases were found in the adventitia and intima. The changes in the adventitia uniformly consisted of perivascular infiltrations of lymphocytes and a varying number of plasma cells about the vasa vasorum, and endarteritis obliterans of the vasa vasorum. This latter change, however, was encountered much more frequently in the vasa vasorum of the larger vessels with a relatively rich elastica than in the smaller vessels with a relatively rich musculature. As suggested in a previous paper, the endarteritis occurs in vessels of a certain caliber only. The vasa vasorum of smaller arteries are smaller, which might be the reason that endarteritis obliterans is found less often in these vessels. From this series we are unable to state definitely which lesion is primarily present, the endarteritis of the vasa vasorum, or the perivascular infiltration. While it is true that perivascular infiltrations were found in the adventitia of the smaller vessels unaccompanied by endarteritis obliterans, yet the larger vessels always showed not only both changes but also an endarteritis obliterans of the vasa vasorum without the perivascular infiltration of lymphocytes. It would be inconsistent to assume that the primary lesion in larger branches of the aorta is an endarteritis obliterans of the vasa vasorum and in smaller branches a perivascular infiltration of lymphocytes. But it is at least safe to assume that the primary stage of syphilis in the branches of the aorta always is found in the adventitia.

The media of a large number of our cases showed an interruption of the elastic lamellae, a new formation of connective tissue and an infiltration of lymphocytes. But whenever these changes were present, the adventitia always showed perivascular infiltration of lymphocytes and endarteritis obliterans. On the other hand, the media in some of the cases was free from changes while the adventitia showed syphilitic lesions. The media of the muscular type of the arteries rarely showed changes. The occasional involvement of

the media in these arteries might easily be accounted for by the rarity of endarteritic changes in the vasa vasorum of the adventitia. If changes were present in the media they were confined to its outer portion, where most of the elastic fibers were present, while the inner portions were free from changes. These findings suggest the propagation of the syphilitic process by way of the vasa vasorum which supply only the corresponding portions of the media.

Throughout the intima diffuse changes characteristic of arteriosclerosis were noted. Fibrosis, hyalinization, calcification, degenerative changes and atheromatous cavities were frequently encountered. In addition to these diffuse changes, circumscribed button-like intimal projections consisting of connective tissue were observed just below the endothelial lining. These lesions were found in the intima at the sites where the adventitia, and in some cases the media, was the seat of a perivascular infiltration of lymphocytes. The diffuse lesions can be interpreted as arteriosclerotic in nature. The severity of these lesions corresponds well with the ages of the patients and substantiates the observations of Hesse,[7] who found severe arteriosclerotic lesions in the arteries of the upper extremities in patients over 40 years of age. The circumscribed, merely proliferative lesions, without degenerative changes, found at the site of syphilitic lesions in the adventitia, however, seem of different etiology. Gurd and Wade [8] believe that syphilis must be recognized as one of the factors leading to proliferation of the intima. Darling and Clark [9] hold that proliferative changes in the intima are a direct response to the syphilitic virus. Whether or not these intimal proliferations really are a manifestation of the syphilitic process cannot be said. Morphologically these lesions show nothing characteristic of syphilis. Stains for spirochetes according to Levaditi and Warthin–Starry did not reveal their presence. There is not enough proof, therefore, to believe that the proliferative changes in the intima are due to syphilis. I am rather inclined to believe that the intimal changes are a non-syphilitic reaction even though brought about by the syphilitic inflammation of the adventitia. The non-syphilitic reaction of the intima probably is a chronic inflammation compensatory to any pathological process in the adventitia, and, in our series, to syphilis.

Conclusions

1. Among fifty cases of syphilitic aortitis, the innominate artery showed syphilitic changes in thirty-three, the carotid in twenty-nine, the superior mesenteric in ten, the inferior mesenteric in three, the common iliac in ten, and the femoral artery in seven cases. The subclavian artery was examined in twenty-nine cases, fifteen of which showed syphilitic lesions.

2. The syphilitic lesions were characterized by endarteritis of the vasa vasorum, and perivascular infiltration of lymphocytes in the adventitia. The media of the elastic type of arteries showed an interruption of the continuity of the elastic fibers and fibrotic areas combined with circumscribed lymphocytic infiltrations. The media of the muscular type of arteries only rarely showed changes.

3. In the intima, circumscribed button-like areas of fibrosis, without degenerative changes, were found very frequently. These are not specific but are chronic inflammatory in nature and might be associated with any type of pathological lesion in the adventitia.

Note: I am deeply indebted to Professor Howard T. Karsner for his valuable suggestions and his aid in taking the photomicrographs, and to Miss Lilian Boker of the Department of Medicine for technical assistance.

References

1. Saphir, O., and Scott, R. W. The involvement of the aortic valve in syphilitic aortitis. *Am. J. Path.*, 1927, iii, 527.
2. Warthin, A. S. Syphilis of the medium and smaller arteries. *New York Med. J.*, 1922, cxv, 69.
3. MacKenzie, I. Syphilis of the circulatory system. *Glasgow M. J.*, 1919, xcii, 209.
4. Lange, F. Studien zur Pathologie der Arterien insbesondere zur Lehre der Arteriosklerose. *Virchows Arch. f. path. Anat.*, 1924, ccxlviii, 463.
5. Letulle, M., Heitz, J., and Magniel, M. Claudication intermittente chez un syphilitique avec lésions de l'aorte, des iliaques et des artères des membres inférieurs. *Arch. d. mal. du coeur*, 1925, xviii, 497.
6. MacCallum, W. G. A Textbook of Pathology. Philadelphia and London, 1928.
7. Hesse, M. Ueber die pathologischen Veränderungen der Arterien der oberen Extremität. *Virchows Arch. f. path. Anat.*, 1926, cclxi, 225.
8. Gurd, F. B., and Wade, H. W. A study of primary intimal arteritis of syphilitic origin. *J. Med. Res.*, 1911, xxv, 85.
9. Darling, S. T., and Clark, H. C. Arteritis syphilitica obliterans. *J. Med. Res.*, 1915, xxxii, 1.

DESCRIPTION OF PLATE

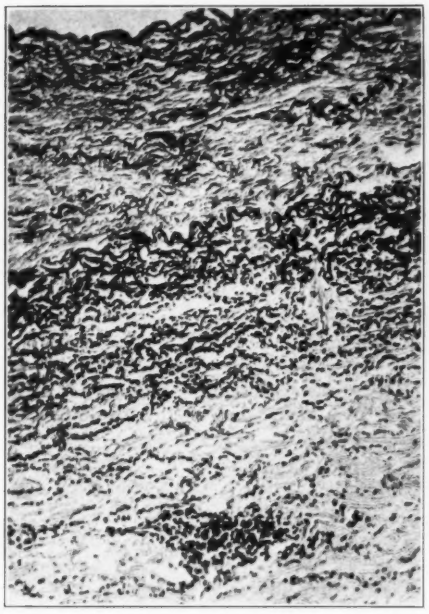
PLATE 85

FIG. 1. Syphilis of the superior mesenteric artery. Note the infiltration of lymphocytes in the adventitia and the inconspicuous interruption of the elastic lamellae in the media. Combination of orcein and iron hematoxylin. × 110.

FIG. 2. Syphilis of the common carotid artery. Note the perivascular infiltration of lymphocytes in the media, and the broken elastic lamellae. Combination of orcein and iron hematoxylin. × 110.

FIG. 3. Gumma of the subclavian artery. Combination of orcein and iron hematoxylin. × 120.

FIG. 4. Syphilis of the superior mesenteric artery. Note the marked intimal reaction. Combination of orcein and iron hematoxylin. × 110.



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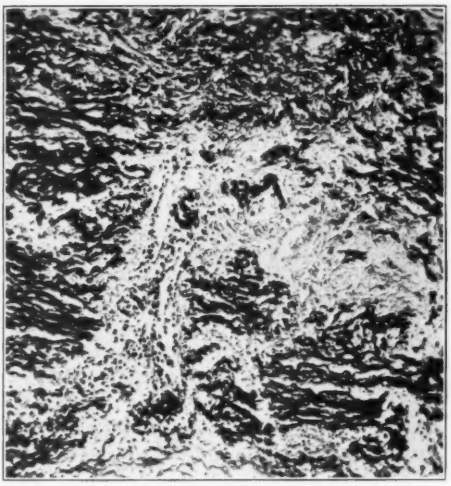

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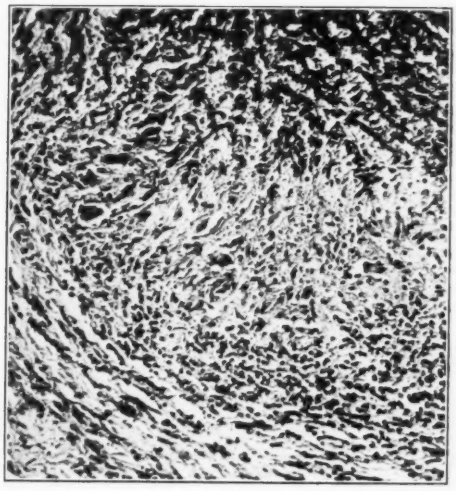

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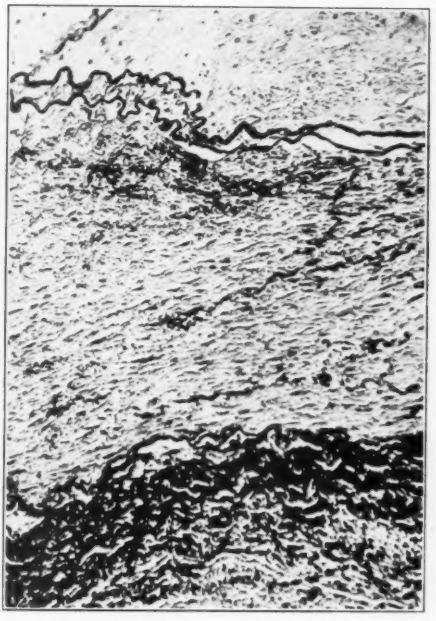

4

Saphir

Syphilis of Medium-Sized Arteries

ACCESSORY PANCREASES; REPORT OF A CASE WITH A DISCUSSION OF THEIR PATHOLOGY AND ORIGIN *

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Not less than one hundred and fifty-five instances of accessory pancreas are reported in the literature, but a clear understanding of their origin has not yet been established. The purpose of this paper is to present a case and to discuss the facts and hypotheses concerning the condition.

REPORT OF CASE

A white female, one year old, well developed and well nourished, was admitted to the service of Dr. H. J. Gerstenberger, Babies and Children's Hospital of Cleveland, Ohio, with expiratory and inspiratory laryngeal obstruction. Tracheotomy was ineffective and death occurred twenty hours after admission.

Autopsy three and one-half hours after death revealed an acute fibrinopurulent inflammation of larynx, trachea and larger bronchi, negative for diphtheria bacilli; pulmonary atelectasis, interstitial emphysema of mediastinum; cloudy swelling of the parenchymatous viscera, fatty metamorphosis of liver, accessory pancreas in wall of ileum and recent tracheotomy wound.

Detailed description will be confined to the stomach and small intestine. The stomach is normal except that the mucous membrane is swollen, hyperemic and covered by a thin mucinous exudate. The duodenum is normal. The jejunum and ileum are normal except for slight dilatation by gas, slight hyperplasia of the lymphatic tissue and a discrete firm nodule, 8 mm. in diameter in the wall, 60 cm. below the duodenal jejunal flexure. The serosa overlying the nodule is thick, white and opaque, but not adherent to surrounding organs. The nodule projects into the lumen but the mucosa is normal. In the mucosa near the center is a small depression through which a

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probe can be introduced into the substance of the nodule. On cut section, the nodule is of a gray-white color and of a firm fibrotic consistency with islands of softer cellular tissue.

Microscopically the mucous membrane is typically jejunal and normal. The serosa is slightly thickened by dense fibrous tissue. The nodule is composed of typical pancreatic acini, islet cells, intercalated ducts (Fig. 2), larger ducts and fibrous tissue. On the serosal side the fibrous tissue is in excess and infiltrated with a few lymphocytes. The acini in this area are isolated and compressed (Fig. 3). One small lymph node in the edge of the nodule is hyperplastic. Cells cytologically identical with islet cells are irregularly distributed in groups of two to ten. The larger ducts are typically pancreatic. The parenchymatous cells do not exhibit evidence of proliferation. The fibers of the tunica muscularis are separated by a loose fibrous tissue and a few pancreatic acini. Near the exit of the ducts is a small circumscribed mass of tissue (Fig. 4) bounded by smooth muscle and enclosing typical pancreatic ducts and other smaller acini with cells similar to those of Brunner's glands. Stains for zymogen granules were unsuccessful, probably due to improper fixation.

Histologic Features

The general structure of these nodules is such that the diagnosis is evident on microscopic examination. The parenchymatous tissues which may be found are pancreatic acini, islands of Langerhans, larger ducts and glands of Brunner.

The pancreatic acini are usually normal in their structure and arrangement. The acini and ducts do not have the appearance of proliferation and in many cases the acinar cells contain zymogen granules.

The presence of islands of Langerhans is variable. In many reports there is no mention of their presence, in others they were identified with difficulty, while in still others, islands in normal number and size are reported. There is no information concerning the function of such islands as have been observed.

Ducts lined with a typical pancreatic duct epithelium are distributed irregularly throughout the pancreatic acini. In many instances these ducts coalesce into one or two which open into the intestine, or rarely into the bile duct as described by Opie,[1] or main

pancreatic duct as reported by Bean and Dreyer.[2] Nicholson [3] found that these ducts may be directly continuous with ducts lined by cells identical with those of Brunner's glands.

The interstitial supporting tissue may be in the usual amount or may show definite increase with cellular infiltration indicative of chronic inflammation, first pointed out as a prominent feature by Opie. Warthin [4] reported areas of fat necrosis.

In the majority of cases the overlying mucous membrane is typical of that portion of the intestine in which the ectopic mass is found, but in a few the overlying mucosa is typically duodenal, whatever the location.

If the ectopic mass occupies the muscularis, the fibers are often separated by pancreatic or fibrous tissue. This observation is of importance in the consideration of diverticula.

The histologic features of the case here reported do not differ markedly from the above general description. Islet cells are difficult of identification, and fibrosis (Fig. 3) is a prominent feature. The continuity of pancreatic duct epithelium and Brunner's gland epithelium can be demonstrated (Fig. 4).

Theories of Origin

Zenker [5] first proposed that ectopic pancreases have their origin in accessory pancreatic anlagen. Gliński [6] postulated non-fusion of the three primary pancreatic anlagen. Warthin modified these theories with the hypothesis that they represent secondary diverticula snared off from the primary anlagen as they pass through the intestinal wall. Lubarsch [7] believed that inflammatory adhesions may form between the pancreas and adjacent organs with later separation of a small piece of pancreatic tissue. Horgan [8] proposed the same, except that non-inflammatory adhesions are formed during fetal life when all the organs are in close approximation. Taylor [9] assumed that they result from heteroplasia of a portion of the fetal entoderm and places them in the class of "dysontogenetic" structures. Finally, Beutler [11] hypothecated that they are atavistic phenomena.

Thyng [11] has given strong support to Zenker's theory by demonstration of accessory pancreatic anlage in two pig embryos. Horgan has objected to Gliński's non-fusion hypothesis on the grounds that all accessory pancreases would be of large size and located in the

duodenum. This is not valid since if there is a defect in migration there is also very probably an associated defect of development. Further, lengthening and growth of the foregut could carry the anlage distant from the duodenum. A serious objection to Gliński's hypothesis is that it does not explain instances of three accessory pancreases, cases of which are cited by Opie. The reports by Opie of annular pancreases demonstrate that non-fusion may occur.

Simpson [12] supports Warthin's hypothesis on the following points: that accessory pancreases are found most frequently about the papilla of Vater, that they never occur outside the derivatives of the foregut, and that they have been found in the omentum by Warthin, and in the gall bladder by Mann [13] and by von Hedry.[14] As we see it, these observations only point to an origin in the duodenum and do not support Warthin's theory any more than that of Zenker or Gliński.

Cases, such as that of Bean and Dreyer, in which the duct of the accessory pancreas communicates with the main pancreas lend support to Horgan's conception of fetal detachment. The usual absence of anatomical approximation and adhesions makes Lubarsch's theory doubtful except in rare instances.

Taylor has attempted to distinguish accessory pancreases from the adenomyoma of the upper gastro-intestinal tract. He considers the adenomyoma to be a true tumor, characterized by the presence of Brunner's glands with few or no pancreatic acini. Such a distinction has confused his heteroplastic hypothesis for the origin of accessory pancreases. Simpson believes that the adenomyoma is an accessory pancreas in which the acini never developed, regressed or were destroyed by inflammation. Our demonstration of Brunner's glands in an accessory pancreas makes us believe that the cells of the accessory pancreatic anlage are potential acinar, islet, duct or Brunner gland cells.

Beutler's postulation of an atavistic phenomenon is consistent with embryology, but the restriction of all reported accessory pancreases to structures derived from the foregut and its mesentery is a serious objection.

From this review our conclusion must be that in different cases different factors operate: in some, inflammatory or embryonal adhesions with subsequent detachments of a mass of pancreatic cells; in others, development from the primary anlage of Zenker or Gliński,

or the secondary snared-off anlage of Warthin, with the added conception that the cells of these anlage are still undifferentiated entoderm and capable of differentiating into pancreatic acini, islands of Langerhans, ductal epithelium and glands of Brunner. We are unable to accept any theory of heteroplasia, neoplasm or atavistic pnenomena.

Complications and Potentialities

Diverticula: Simpson states that twenty-five of the one hundred and fifty cases of accessory pancreases collected by him were in diverticula. This association is of more than passing interest because of the cause-and-effect relation between the two. From a study of microscopic sections of an accessory pancreas in the intestinal wall, the separation of the muscle bundles leads to the supposition that the wall is weakened at this point and that the diverticulum is secondary to the congenital anomaly. Neumann[15] first proposed such a relationship. Simpson and others support it, placing these cases in the class of true pulsion diverticula. That an accessory pancreas can occur in a true Meckel's diverticulum is undisputed. Koch and Suzuki[16] made a systematic study of fifty cases of Meckel's diverticulum and found accessory pancreatic tissue in two. The finding by Wright[17] of an accessory pancreas in a congenital umbilical fistula, proves such a probable origin.

Since an ectopic pancreas has not been observed in the large intestine, it is unlikely that diverticula here are due to this condition, as suggested by Gibson.[18]

Invagination and Intussusception: When these tumors are located in the submucosa they may occasionally become pedunculated and hang into the intestinal canal as polyps. According to the reports of Hulst[19] and Brunner[20] the intestinal wall was definitely invaginated. Simpson has admirably summed up the whole situation in regard to these processes. He believes that diverticula and invagination are closely related processes, and that which occurs depends entirely on the location of the mass, the amount and character of intestinal peristalsis and the interruption of the muscularis by the mass. Benjamin[21] reports a case of intussusception in which an accessory pancreas was found in the wall of the intussusceptum. Opie and others observed frequent inflammatory changes in these masses, which might well serve as the direct exciting factor of irreg-

ular peristalsis in producing the intussusception. The whole problem appears to be worthy of further study.

Congenital Pyloric Stenosis: Hale [22] reports a small bit of pancreatic tissue embedded in the hypertrophic musculature of a case of congenital pyloric stenosis. Since it had no visible duct, he drew the conclusion that the presence of the ectopic tissue plus its retained secretion led to hypertrophy of the muscle, and suggests that all congenital pyloric stenosis is due to this underlying condition. That a ductless pancreas would secrete and that the pancreatic enzymes would cause only a hypertrophy of muscle is inconsistent with experimental and autopsy observations.

Neoplastic Transformation: Several problems are involved in the relation of these congenital defects to neoplasms. The most important practical consideration is the differential diagnosis at operation. In the case reported by Choisser [23] a small tumor 1.5 cm. by 2 cm. in the wall of the pyloric antrum with enlarged and firm mesenteric lymph node was encountered in a man 57 years of age, complaining of pain in the epigastrium and loss of weight. In instances of this type the differentiation would be extremely difficult. In general an ectopic pancreas is a small round discrete mass in the structures derived from the foregut, and rarely over 1 cm. in diameter.

The relation of ectopic pancreases to adenomyomata has been discussed. Trappe [24] proposed that the carcinoids of the small intestine arise as neoplasms from pancreatic rests, while Saltykow [25] limited their origin to rests composed entirely of islands. The most serious negative objection to this is that carcinoids are most frequent in the appendix, while an accessory pancreas has never been reported in this location. In addition there is the evidence of Masson [26] that the carcinoids of the intestine arise from the chromaffin cells of the crypts of Lieberkuhn.

Finally, there is the question of malignant change in an ectopic pancreas. Branham [27] reports a case in which Welch made such a diagnosis. Nicholson reports another occurring in the stomach and cites two similar cases by Herxheimer [28] and by Seidelin.[25] Although these four cases of carcinoma and one hundred and fifty of ectopic pancreas probably do not represent the total number which have been observed, it would appear that the incidence of carcinoma is higher in ectopic pancreases than in the normal pancreas.

Summary

1. A case of accessory pancreas in the wall of the ileum in a one-year-old child is reported.

2. The mode of origin of these congenital anomalies is either by adhesions of the main pancreatic anlage or from anomalous anlage.

3. Accessory pancreases are in rare instances the underlying cause of diverticula and intussusception and the site of origin of carcinoma.

NOTE: I wish to thank Dr. Howard T. Karsner for valuable suggestions in the writing of this paper and for aid in the preparation of the photomicrographs. I also acknowledge the courtesy of Dr. H. J. Gerstenberger for the use of the clinical material.

REFERENCES

1. Opie, E. L. Disease of the Pancreas, its Cause and Nature. Lippincott, Philadelphia, 1903.
2. Bean, R. J., and Dreyer, N. B. Duplication of pancreatic bladder and accessory pancreas in the cat. *Anat. Record*, 1927, xxxvi, 155.
3. Nicholson, G. W. Studies on tumour formation. V. The importance of congenital malformations in tumour formation. *Guy's Hosp. Rep.*, 1923, lxxiii, 37.
4. Warthin, A. S. Two cases of accessory pancreas (omentum and stomach). *Physician and Surgeon*, Detroit and Ann Arbor, 1904, xxvi, 337.
5. Zenker, F. A. Nebenpankreas in der Darmwand. *Virchows Arch. f. path. Anat.*, 1861, xxi, 369.
6. Gliński, L. K. Zur Kenntnis des Nebenpankreas und verwandter Zustände. *Virchows Arch. f. path. Anat.*, 1901, clxiv, 132.
7. Lubarsch, O. Ueber das Vorkommen von Pankreasläppchen in der Milz. *Virchows Arch. f. path. Anat.*, 1925, ccliv, 880.
8. Horgan, E. J. Accessory pancreatic tissue, report of two cases. *Arch. Surg.*, 1921, ii, 521.
9. Taylor, A. L. Epithelial heterotopias of the alimentary tract. *J. Path. & Bact.*, 1927, xxx, 415.
10. Beutler, A. Ueber blastomatöses Wuchern von Pankreaskeimen in der Magenwand. *Virchows Arch. f. path. Anat.*, 1921, ccxxxii, 341.
11. Thyng, F. W. Models of the pancreas in embryos of the pig, rabbit, cat and man. *Am. J. Anat.*, 1907–08, vii, 489.
12. Simpson, W. M. Aberrant pancreatic tissue; analysis of 150 human cases with report of a new case. *Warthin Ann. Vol.*, Ann Arbor, 1927, 435.
13. Mann, F. C. An accessory pancreas in the wall of the gall bladder of a dog. *Anat. Record*, 1922, xxiii, 351.
14. von Hedry, N. Beitrag zur Klinik des accessorischen Pankreas. *Beitr. z. klin. Chir.*, 1923, cxxx, 349.
15. Neumann, E. Nebenpankreas und Darmdivertikel. *Arch. d. Heilkunde*, 1870, xi, 200.

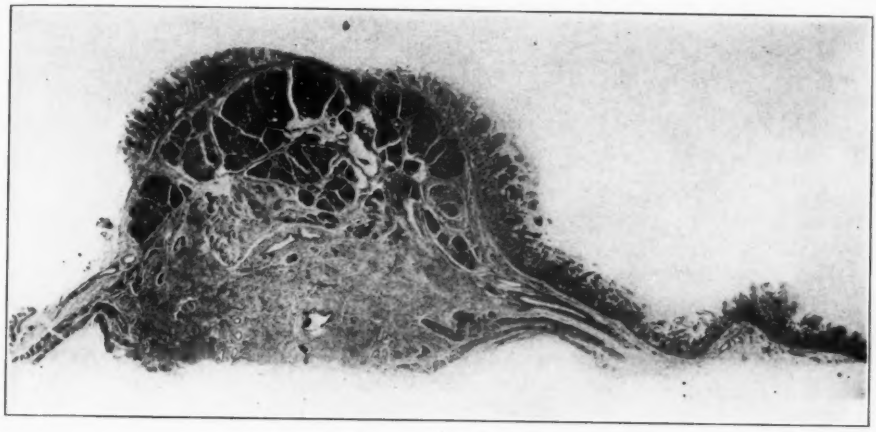
16. Koch, M., and Suzuki, T. Zur Histologie des Meckelschen Divertikels. *Centralbl. f. allg. Pathol. u. path. Anat.*, 1912, xxiii, 904.
17. Wright, J. H. Aberrant pancreas in the region of the umbilicus. *J. Boston Soc. Med. Sc.*, 1901, v, 497.
18. Gibson, C. L. Accessory pancreas in the gastro-intestinal tract. *M. Rec.*, 1912, lxxxii, 426.
19. Hulst, J. P. L. Ueber einen in einem Darmdivertikel gelagerten Pancreaskeim mit sekundärer Invagination. *Centralbl. f. allg. Pathol. u. path. Anat.*, 1909, xx, 12.
20. Brunner, C. Ein Beitrag zur Chirurgie und pathologischen Anatomie der Darminvagination. *Beitr. z. klin. Chir.*, 1899, xxv, 344.
21. Benjamin, A. E. Accessory pancreas with intussusception. *Ann. Surg.*, 1918, lxvii, 293.
22. Hale, K. A study of the accessory pancreas; with report of one causing congenital pyloric stenosis. *Ann. Surg.*, 1926, lxxxiii, 774.
23. Choisser, R. M. Aberrant pancreas of pylorus, with report of a case resembling a neoplasm. *U. S. Nav. M. Bull.*, 1925, xxii, 322.
24. Trappe, M. Ueber geschwulstartige Fehlbildungen von Niere, Milz, Haut und Darm. *Frankfurt. Ztschr. f. Path.*, 1907, i, 109.
25. Saltykow, S. Ueber die Genese der "karzinoiden Tumoren" sowie der "Adenomyome" des Darmes. *Beitr. z. path. Anat., u. z. allg. Pathol.*, 1912, liv, 559.
26. Masson, P. Carcinoids (argentaffin-cell tumors) and nerve hyperplasia of appendicular mucosa. *Am. J. Path.*, 1928, iv, 181.
27. Branham, J. H. Operative treatment of cancer of the stomach, with report of six partial gastrectomies. *Maryland M. J.*, 1908, li, 144.
28. Herxheimer, cited by Nicholson. *Guy's Hosp. Rep.*, 1923, lxxiii, 37.
29. Seidelin, H. Carcinoma of jejunum from aberrant pancreas germ. *J. Path. & Bact.*, 1912–13, xvii, 428.

DESCRIPTION OF PLATES

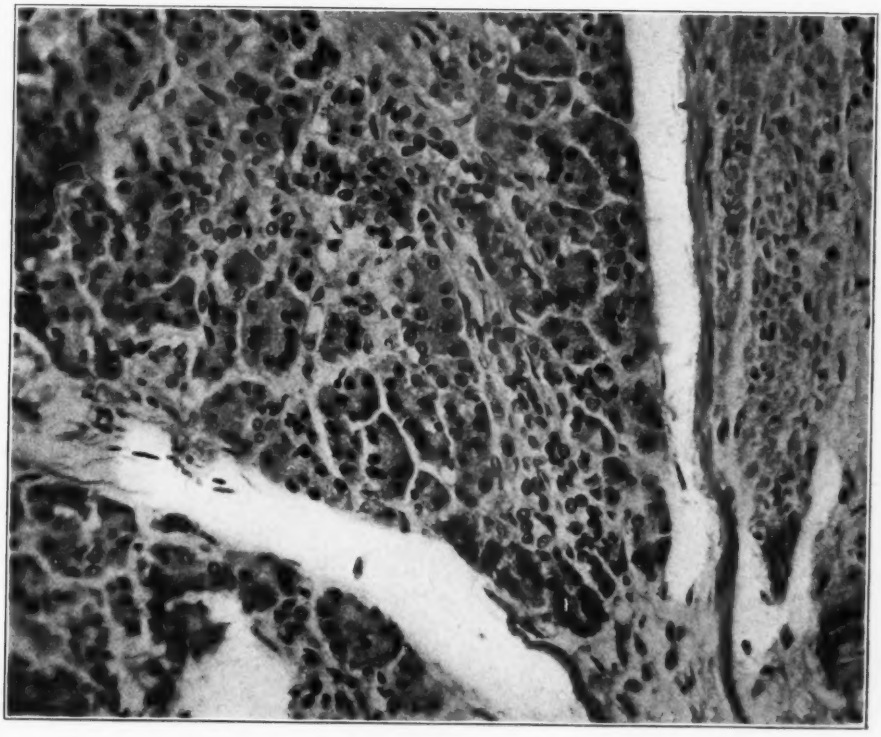
PLATE 86

FIG. 1. A section through the center of the nodule to show the general location and features. Note the fibrosis and relative absence of acini toward the peritoneum. × 8.5.

FIG. 2. Pancreatic tissue to show pancreatic acini, intercalated ducts and small groups of islet cells. × 100.



1

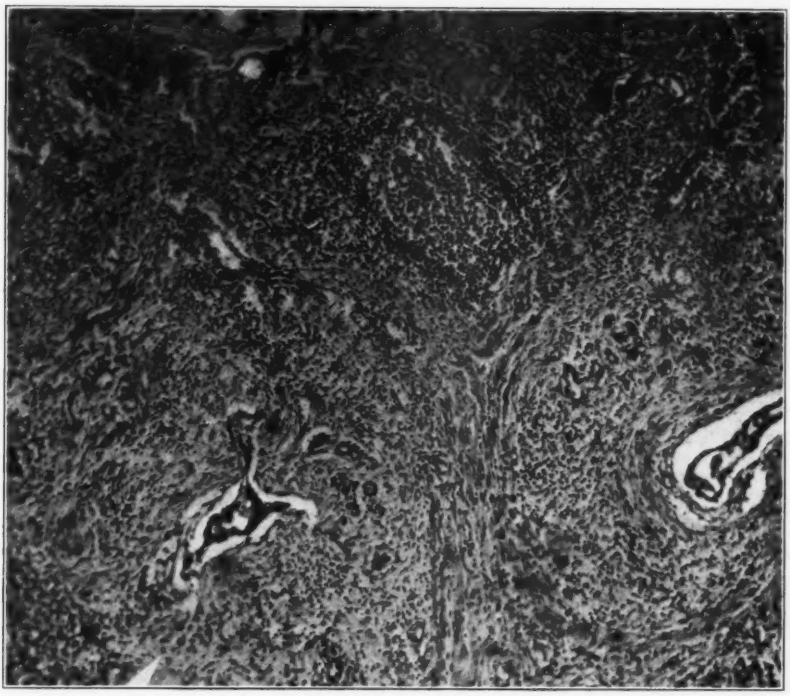


2

PLATE 87

FIG. 3. An area near the peritoneum to show the marked inflammatory reaction, destruction of acini and fibrosis. Note the hyperplastic lymph node. × 100.

FIG. 4. Nodule near the exit of the ducts. Pancreatic duct epithelium is directly continuous with cells of Brunner's gland type. × 110.



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4

THE EFFECT OF POTASSIUM IODIDE, THYROID EXTRACT AND ANTERIOR PITUITARY EXTRACT UPON REGENERATION AND EARLY COMPENSATORY HYPERTROPHY OF THE THYROID GLAND *

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In his studies upon compensatory hypertrophy of the thyroid gland in the guinea pig Loeb has shown that the administration of KI acted in certain respects in an opposite manner to the administration of anterior pituitary extract and thyroid extract.[1, 2, 3, 4, 5, 6, 7] These two latter substances produced a definitely histological inactive gland;[3, 4, 6] the colloid was hard, the epithelium was relatively low and the phagocytic invasion of the colloid was minimal. Potassium iodide,[2, 5, 7] on the other hand, not only did not prevent compensatory hypertrophy but on the average the KI-fed guinea pigs had glands in which the epithelium was taller, the colloid was softer and the phagocytes more numerous than in the control animals. The degree of hypertrophy of epithelial cells was found to be very variable in the controls as well as in the guinea pigs to which KI had been administered; but generally speaking the degree of the hypertrophy was statistically found to be greater in the KI than in the control series.[2, 5, 7] The number of animals used in each of these KI groups was large. Among the variable factors active in these experiments Loeb mentions especially seasonal changes [5] and changes in weight [7] of the guinea pigs during the course of the experiments. Loeb removed one and one-half lobes of thyroid in his animals, because if he removed less, compensatory hypertrophy did not occur even in normal guinea pigs.[1] Most of his experiments were allowed to run twenty and thirty days.

The purpose of this paper is to report the result of the effect of KI, anterior pituitary extract and thyroid extract upon the regeneration and the compensatory hypertrophy of the thyroid gland of guinea pigs from whom the remnants of thyroid had been re-

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moved from five to eighteen days after operation. In contradistinction to the experiments of Loeb this work is concerned with the early stages of hypertrophy. A quantitative estimation of the mitoses was made in addition to the other usual examination of of the gland.

Experiments

Material and Method: Fifty-one guinea pigs, weighing between 350 and 500 gm., were used. One and one-half lobes of the thyroid gland were removed from each of the animals. In removing the half of the lobe, after the thyroid had been freed from the tissue about it, especially from the artery which sometimes is closely applied to it, the lower pole was clamped and the gland cut through the middle. As a rule there was very little bleeding. The use of a ligature would have interfered with the study of regeneration. The morning after operation the animals were divided into four groups. One of the groups received no treatment, acting as the subjects for the study of normal compensatory hypertrophy and as controls for the other three groups that were fed daily 0.05 gm. of KI, or 5 gr. of anterior pituitary substance (Armour & Co.) or 0.1 gr. thyroid substance (Armour & Co.). The animals were killed by chloroform and their thyroid remnants immediately removed and fixed in Zenker from five to eighteen days after the beginning of treatment. The thyroid remnants were sectioned serially and mounted in rows of eight, nine, or ten. The mitoses in one section in each row were counted and the number thus obtained multiplied by the number of sections in the row. Thus the number of mitoses was obtained for the entire remnant.

Gross Findings: The only observation in the gross specimens of any interest was the greater size and perhaps redder color of the remnants removed from the control and the KI-fed animals as compared to the remnants removed from the anterior pituitary and thyroid-fed guinea pigs.

I. Regeneration

At the site of incision into the thyroid a definite process of regeneration of the acini could be studied. In the earlier glands examined the tissue in this region consisted either of small groups of thyroid cells that had no definite acinar arrangement, or of many very small

acini lined by deeply staining cuboidal epithelium; some of the acini contained tiny droplets of colloid, while others did not. There also were larger irregular acini usually without colloid but containing many polymorphonuclear leucocytes or phagocytic cells. The small acini were formed in three ways: by the reunion of the remaining cells of a partially destroyed acinus; by the differentiation of the small groups of epithelial cells; and by the growth of connective tissue into a larger acinus and the subsequent division of this acinus. The stage that follows this, which is denoted by the presence of large acini, irregular in shape with rather tall epithelium and soft or no colloid, is brought about chiefly by proliferation of the epithelial cells. The greater number of mitoses in the areas of regeneration than in the other parts of the gland supports this view. In the glands removed at five, seven, eight and nine days most of the mitotic activity of the epithelium was found in this region. The return of these acini to the type of acini in the adjacent portions of the thyroid gland so that there was an unnoticeable transition between the two was the final stage.

In the untreated animals regeneration occurred fairly rapidly so that by the tenth and eleventh day the areas of regeneration were becoming imperceptible. The thyroid of the animals fed with KI regenerated about as rapidly as the controls, the eleventh and twelfth days being periods when the return to normal was becoming definite. It should be mentioned that an eight-day control gland and one of the nine-day KI glands had regenerated so rapidly as to resemble the picture that becomes more commonly pronounced on the twelfth day. The glands of the animals fed with anterior pituitary and thyroid never approached in our experiments the amounts of regeneration noted in the controls and KI series, so that even in the latest examined periods, regeneration was not complete in the thyroids of these animals. This retardation of regeneration was more marked in the animals fed with thyroid than in those fed with pituitary.

II. Compensatory Hypertrophy

Mitotic Activity: In Table II the number of mitoses found in the remnants of each gland is tabulated. Marked proliferative activity of the acinar epithelium is indicated in the glands of the untreated animals and in the glands of the KI-fed animals, setting these two

groups definitely apart from the glands of animals fed with pituitary extract and thyroid extract. The average numbers of the mitoses are 1105, 763, 206 and 70, respectively. If we do not include the figures obtained in the thyroid remnants removed at the earliest periods, when the effect of the substances fed may not have become manifest and when most of the proliferative activity is confined to

TABLE 1

Mitotic Activity in Thyroid Remnant

No. of days	Control	KI	Pituitary	Thyroid
5	527*	291*	453*	...
7	121*	...	242*	83*
8	0*	758	298	152
9	1158	96	220	228
9	...	308	210	72
10	1752	1612	138	0
11	1108	995	104	...
11	348	67	36	...
12	4560	816	486	40
13	1368	764	82	46
14	...	...	...	92
15	490	3217	352	0
15	0	880	0	0
16	200	27	54	60
18	2959	96	...	...
18	887	...	...	...
Average of all	1105	763	206	70
Omitting *	1348	803	180	69

the areas of regeneration, our averages of the mitoses are 1348, 803, 180 and 69. The variability in the response of the thyroid gland of the guinea pig to the administration of KI is shown by the wide range of the number of mitoses recorded: 3217 and 27.

The mitotic activity in KI administration is not caused solely by the stimulating effect of KI upon the thyroid gland that has been observed by Gray and Loeb,[8] Rabinovitch,[9] and McCordock,[10] but is also caused by the compensatory hypertrophy. In the KI and control animals the remnants at the time of examination were approximately two-thirds the size of the normal thyroid lobe, and in

the guinea pigs fed anterior pituitary or thyroid substance, the remnants were approximately one-half the size of the normal lobe. The figures obtained in the former may therefore be multiplied by three, and those obtained in the latter by four, in order to compare the number of mitoses in compensatory hypertrophy with the number of mitoses found in the unoperated normal guinea pigs. Thus we obtain the figures 4044, 2409, 720, 276 for the controls, KI, pituitary extract and thyroid-fed animals respectively. The average number of mitoses for these KI compensatory hypertrophy animals as compared to unoperated KI animals is over three times the amount obtained by Gray and Loeb,[8] twice that obtained by McCordock,[10] seven times that obtained by Rabinovitch [9] on the tenth day of his experiments, but it is less than the number obtained by Rabinovitch on animals fed with 0.05 gm. KI daily for fifteen days. In this last series Rabinovitch used only two guinea pigs and the figures may be somewhat high. In pituitary feeding and thyroid feeding, the number of mitoses is only very slightly higher in animals in which a part of the thyroid was extirpated than in normal animals.[8, 10] In the animals that received thyroid and pituitary substance, regeneration may partially account for the increased number of mitoses as compared to normal animals, especially in those fed with thyroid. In the pituitary animals, however, there appears to be a slight definite effect produced through compensatory hypertrophy.

There is another very marked difference between the number of mitoses in the iodized guinea pigs and in those treated with anterior pituitary or thyroid substance. In the latter class the number of mitoses is fairly constantly low, whereas in the animals which received KI, there is very marked variation in the number of mitoses in different cases, similar to the variations found in controls. This indicates that variable factors enter and complicate the degree of hypertrophy observed in control and iodized guinea pigs. These will be discussed later.

Size of the Acini: No exact measurements of the size of the acini were made, but in general we can state that approximately the size of the acini was about the same in the controls and KI-fed animals, and that it was smaller in pituitary and thyroid-fed animals.

Size of the Epithelium and the Character of the Colloid: There exists in general a direct parallelism between the height of the epithelium and the consistency of the colloid, except that the increase in the

size of the epithelium was noted a little earlier than the first signs of the softening of the colloid. In the thyroids of the animals fed with thyroid substance no increase in the size of the epithelium and no softening of the colloid were observed. The epithelium here was low cuboidal and the colloid hard, with occasional retraction; only rarely peripheral vacuolization was noted. In the thyroids of those animals which were fed with anterior pituitary substance the epithelium was a little higher and the colloid a little softer than in the guinea pigs fed with thyroid substance. This applies to periods following approximately the tenth or eleventh day, but this difference was slight. In the animals fed with KI and in the control animals definite signs of compensatory hypertrophy were noted on the eighth and ninth days. At this time the epithelium in both approached in some cases the columnar shape and later became definitely columnar; and the colloid in both showed peripheral vacuolization, became soft and even disappeared. In our series of animals the thyroid remnants of the control guinea pigs showed, on the average, slightly more hypertrophy than the remnants of the guinea pigs fed with KI. However, the thyroid glands of two of the KI-fed guinea pigs were more hypertrophic than those of the control thyroids. Again the variation in both series was very great in different cases. In some animals the hypertrophy was slight or absent while in others it was very marked. We found some gland remnants in which the epithelium was high and the colloid soft, in which there was therefore distinct hypertrophy present, although the number of mitoses was low. Usually an increased number of mitoses was associated with the signs of hypertrophy, but rarely exceptions were found for this rule.

Phagocytosis and Lymphocytosis: We can confirm the observations made previously [8] that the number of phagocytic cells in the colloid is greater in animals that have been fed with KI than in the animals of any of the other series. In the control series the acini contained more phagocytes than in the pituitary series, while the colloid in the glands of animals fed with thyroid was invaded by very few phagocytic cells.

No constant relationship between the presence or amount of lymphocytes and the substance fed to the animals could be detected.

Discussion

The delayed regeneration of the incised thyroid gland caused by the administration of anterior pituitary and thyroid substances which we have described might be expected from the previously observed inhibiting effect of these substances upon the activity of both the normal thyroid and upon the thyroid during the process of compensatory hypertrophy as indicated by structural changes. Thus we now find that these two substances produce not only a histological and probably a functional resting stage, but also by their depressing effect upon the proliferative activity of the acinar epithelium cause a delayed regeneration in the gland. Because of the known stimulating effect of KI upon the mitotic activity of the normal thyroid gland, one would suppose that regeneration would occur earlier in the KI-fed guinea pigs than in the control guinea pigs during the process of compensatory hypertrophy. However, the number of animals examined by us at a period between five and nine days is too small for an accurate determination of this question.

As far as compensatory hypertrophy is concerned there can be no doubt, in these experiments at least, that there are marked differences in regard to the number of mitoses, the height of the epithelium and the consistency of the colloid between the thyroid glands of animals fed with anterior pituitary and thyroid extracts on the one hand, and the thyroid glands of animals fed with KI and the controls on the other hand. The former gave the histological picture of resting glands, the latter gave the histological picture of definite hypertrophy.

Taking the series as a whole, the thyroids of the control guinea pigs underwent slightly more epithelial proliferation and hypertrophy and slightly more colloid softening than the thyroids of those animals fed with KI. It must be remembered, however, that there was variation in the animals in each series, possibly somewhat more in the KI animals. These individual differences in intensity of compensatory hypertrophy in normal as well as in iodized guinea pigs has been emphasized before by Loeb.

Among the variable factors responsible for these individual differences in the degree of compensatory hypertrophy the change in weight of the animals during the progress of the experiment is very important. As Loeb, and Rabinovitch [11] have shown, animals that

gain in weight tend to show a more marked hypertrophy and a greater proliferation of the normal gland than animals that remain stationary or that lose weight during the time of the experiment. In our series ten of the controls gained in weight, four remained stationary and none lost weight, while in the KI series, three of the animals lost weight, while four remained approximately stationary and only six gained in weight. This may explain why in our series the average of compensatory hypertrophy was somewhat greater in the control series.

Furthermore, it is possible that the action of KI upon the thyroid gland does not reach its maximum until after sixteen to eighteen days, which is the period of marked proliferation in normal unoperated guinea pigs as the experiments of Gray and Loeb, and Rabinovitch indicate. Considering the importance of these variable factors the number of animals used by us in the control and KI series is not yet large enough to determine whether the compensatory hypertrophy in the early periods following removal of a great part of the thyroid gland is somewhat greater in the controls than in the iodized animals. On the other hand, we can state that even in these early periods administration of anterior pituitary as well as thyroid substance prevents compensatory hypertrophy, while KI does not have such an inhibitory effect.

Conclusions

1. Regeneration of thyroid tissue after an incision occurs rapidly in control and KI-fed animals.

2. Regeneration is delayed by the feeding of pituitary extract and thyroid extract.

3. Early compensatory hypertrophy is markedly inhibited by pituitary extract and thyroid extract but only slightly by KI.

4. The possible causes of a slight diminution in the average hypertrophy in the KI series which we observed at early periods, are discussed.

5. Compensatory hypertrophy may appear as early as eight days following the removal of parts of the thyroid glands.

REFERENCES

1. Loeb, Leo. *J. Med. Res.*, 1919, xl, 199.
2. Loeb, Leo. *J. Med. Res.*, 1920, xli, 481.
3. Loeb, Leo. *J. Med. Res.*, 1920, xlii, 77.
4. Loeb, Leo, and Kaplan, E. E. *J. Med. Res.*, 1924, xliv, 557.
5. Loeb, Leo. *Am. J. Path.*, 1926, ii, 19.
6. Loeb, Leo. *Am. J. Path.*, 1929, v, 71.
7. Loeb, Leo. *Am. J. Path.*, 1929, v, 79.
8. Gray, S. H., and Loeb, Leo. *Am. J. Path.*, 1928, iv, 257.
9. Rabinovitch, J. *Am. J. Path.*, 1928, iv, 601.
10. McCordock, H. A. *Am. J. Path.*, 1929, v, 171.
11. Rabinovitch, J. *Am. J. Path.*, 1929, v, 87.

AUTO- AND HOMOIOTRANSPLANTATION OF CROSS-STRIATED MUSCLE TISSUE IN THE RAT*

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The aim of these experiments has been, in the first place, to analyze the difference between autotransplantation and homoiotransplantation of cross-striated muscle and, secondly, to study the changes which the muscle pieces undergo at various periods following transplantation.

The literature on muscle transplantation is extensive, but the large majority of these experiments were carried out from surgical points of view; they concerned mainly autotransplantation. Some authors found that the transplanted muscle tissue became completely necrotic soon after transplantation; others found that it showed peripheral regeneration for some time and then degenerated, and still others concluded that it remained alive *in toto*, especially if nerve connections were soon established between host and transplant. It will not be necessary to review here the literature in detail; instead, we can refer to the review of Eden in Lexer's [1] book on transplantation. For our purposes it will be sufficient to refer specifically to the experiments of Saltykow,[2] who carried out an extensive series of autotransplantations in rats and mice, of Askanazy [3] and Jores–Köln,[4] and of Shinya,[5] the only author who compared carefully the results of auto- and homoiotransplantation of striated muscle tissue.

Concerning the differences between auto- and homoiotransplantation in general, Loeb [6] and his associates studied auto-, syngenesio- and heterotransplants of various organs. In a summary of his own investigations and of those of his collaborators, he concludes that the reactions against the transplant are quantitatively parallel to the relationship between host and donor and that the behavior of the lymphocytes especially, but also of connective tissue, can be

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used as a quantitative indicator of that relationship. The autotransplants have the greatest degree of efficiency in preserving their integrity, in maintaining inviolate the relationship between parenchyma and stroma and in preventing the invasion of the transplant by lymphocytes and connective tissue of the host. This efficiency decreases as we pass from syngenesio- to homoiotransplants. The lymphocytic reaction is lacking in the autotransplant and it increases progressively as the relationship between host and transplant becomes more distant, reaching a maximum in the homoiotransplant. It decreases in the heterotransplant if the toxic action of the body fluids has caused a marked injury of the transplanted tissue; otherwise the reaction is very pronounced in these cases and, in addition to lymphocytes, polymorphonuclear leucocytes may be attracted.

Saltykow made free autotransplantations of muscle and observed that, at first, the muscle degenerated. At five days he found evidence of far advanced regeneration, the muscle nuclei being now more numerous and larger in size. The contractile substance surrounding the nuclei degenerated into small, granular masses and disappeared. Gradually there occurred a formation of spindles and from these, narrow muscle fibers were produced. He also observed that the fibers closest to the periphery were either best preserved or showed the greatest regeneration, owing to their greater proximity to the blood vessels of the surrounding tissue. At two weeks the fibers were wider and the nuclei, although still increased in number, were situated in the periphery of the fibers and had become less numerous. A few fibers showed suggestions of cross-striations. At four weeks, the new fibers differed from the original ones only by being a little narrower. During the later stages a simple atrophy took place. At the beginning of the fourth month scarcely any cross-striated muscle fibers remained. He explains the final atrophy as being due to lack of use of the transplanted tissue.

Askanazy transplanted muscle into the brain and observed regeneration at the end of the first week, but at the beginning of the third week he found signs of atrophy in most of the newly formed fibers. At two months, only the last remnants of the transplanted muscle fibers showing a fibrillar structure were seen. According to Jores, faradization of the autotransplanted muscle piece increases the regeneration of the peripheral muscle fibers and the temporary preservation of parts of the transplants.

Shinya was the only author who, besides studying the fate of the muscle transplants, also compared the histological pictures resulting from auto- and homoiotransplantation of cross-striated muscle into peripheral nerves. He concluded that muscle can be transplanted to the same host as well as to unrelated hosts of the same species with good results. In accordance with the previous findings of Loeb and his collaborators, Myer [7] and Sale,[8] in the case of other organs, Shinya observed that the lymphocytic reaction is much stronger in the homoio- than in the autotransplant, and also that the muscle fibers maintain themselves much longer in the latter than in the former. Furthermore, the intensity of new formation of muscle fibers was much less and the newly formed fibers degenerated much earlier in the homoio- than in the autotransplant. After thirty-five days he found that the autotransplanted muscle had disappeared completely and had been replaced by connective tissue. In the homoiotransplants, at twenty-seven days there was merely a necrotic dark red-staining piece visible, in which many leucocytes had accumulated. Shinya used dogs for his experiments and in transplanting the tissue he made a longitudinal split in the perineurium of the sciatic nerve, through which a piece of muscle was transferred into the middle of the nerve substance; the perineurium was afterwards sewed up with human hair to prevent the extrusion of nerve substance and of the transplant.

Concerning the methods by which the cross-striated muscle fibers regenerate, different views have been expressed. Some authors state that the regeneration occurs through outgrowth of cells of an embryonal type which later produce muscle tissue, while others maintain that it takes place by means of terminal and lateral bud formation in the cut muscle fibers. According to Krause [9] the nuclei of the old muscle fibers increase and around these the muscle cells form. Saltykow, in his experiments, never observed a genuine budding of the old contractile substance into young fibers. Shinya concluded that the manner of formation of new muscle fibers differs in the auto- and homoiotransplants. In the former there were seen mainly single sarcoblasts and cellular chains of syncytial elements, while in the latter the multinuclear buds of muscle were predominant. According to this author, this difference is a morphological expression of the biochemical differences between individuals of the same species.

Experiments

In studying the problems above stated, we used rats in which we excised a piece of the xyphoid cartilage, together with a part of the cross-striated muscle attached to it, and then transplanted it either into a subcutaneous abdominal pocket of the same rat (autotransplant) or of another unrelated rat (homoiotransplant). Rats from different breeds were used to ensure lack of relationship in case of homoiotransplantation. In every experiment each animal received both an auto- and a homoiotransplant in two different subcutaneous pockets, from which the pieces were removed after varying periods ranging from two days to one hundred eighteen days. In the main our observations were as follows:

Two-Day Autotransplant: The muscle fibers are almost everywhere degenerating, most of them having undergone necrosis. A few of the fibers still have cross-striations. Many polymorphonuclear leucocytes are invading and destroying the muscle. Small blood vessels are sometimes seen among the muscle fibers.

Two-Day Homoiotransplant: The appearance of the muscle is similar to that seen in the two-day autotransplant. Perhaps the fibers are slightly more fibrillar and granular in the homoiotransplant, but this difference is probably without significance.

Four-Day Autotransplant: The muscle, for the most part, is broken up and degenerated. Cross-striations can still be seen in a few degenerating fibers; the large majority of the nuclei are pyknotic. Peripherally, the muscle tissue is being replaced by fibroblasts, round cells and a few small blood vessels. However, in this area there are a few small slender muscle spindles which are multinucleated, the nuclei being vesicular. There are also present in the periphery, small circular remnants of muscle tissue cells containing much cytoplasm and showing multiple nuclei. Thus we find in this piece, associated with degenerative processes in the muscle fibers, proliferation of muscle nuclei.

Lymphocytes are numerous in the periphery and they have invaded and destroyed the muscle fibers in that region. Centrally, although the fibers are almost everywhere degenerated, there is very little lymphocytic infiltration.

Four-Day Homoiotransplant: Most of the muscle appears normal, although occasionally a necrotic fiber is seen. A few of the muscle

nuclei are pyknotic. There is here, little or no evidence of regeneration such as was seen in the four-day autotransplant. Peripherally there is a slight increase in the number of lymphocytes and fibroblasts.

Six-Day Autotransplant: In this piece there is almost everywhere very marked proliferation of the muscle nuclei. The transplant is made up chiefly of long slender muscle spindles which stain bluish pink and contain numerous vesicular nuclei. Very faint cross-striations can be seen in many of the spindles. Nucleoli are present in a number of the muscle nuclei. In a few of the muscle spindles the nuclei are clumped together at one end. Peripherally, many remnants of muscle tissue, small in size, round in shape and containing multiple vesicular nuclei are present. There are a moderate number of lymphocytes and connective tissue cells scattered diffusely between the spindles, invading and replacing some of them. Small blood vessels are also seen among the lymphocytes and connective tissue cells. No budding off of new muscle fibers from the old can be observed.

Six-Day Homoiotransplant: A great part of this transplant is made up of small, circular, multinucleated muscle remnants. There are seen also a number of slender pink-staining muscle spindles containing multiple large vesicular nuclei. However, the muscle spindles do not appear to be as numerous here as in the autotransplant, nor is the degree of nuclear proliferation as intense. No mitoses can be found. Very faint cross-striations are present in a few of the multinucleated spindles. Lymphocytes are seen almost everywhere in the transplant, the infiltration with these cells being much greater here than in the autotransplant. Many of the lymphocytes have, among them, large vesicular nuclei which are surrounded by only a very small amount of cytoplasm. There are, furthermore, scattered fibroblasts as well as small blood vessels throughout the transplant.

Ten-Day Autotransplant: At one end of the transplant is an area in which are seen moderately long, pink-staining muscle spindles which are multinucleated, the nuclei being situated, not in the center as formerly, but chiefly at the periphery of the spindles. In previous transplants the nuclei in the spindles were more numerous and vesicular and a larger number of them were situated centrally. Thus there is an indication that the muscle fibers are again attaining their

mature state; a large number correspondingly show very distinct and regular cross-striations. In the periphery of the transplant many muscle remnants are present and some round cells, as well as fibroblasts, are invading the muscle tissue leaving many muscle nuclei surrounded by small amounts of cytoplasm. Small blood vessels are also numerous in this area.

Ten-Day Homoiotransplant: The muscle is largely degenerated. In the central part of the transplant there are a few thick degenerated muscle fibers which still have distinct cross-striations. These fibers appear mature and represent, probably, the originally transplanted fibers which now have undergone degeneration. At the ends of several muscle fibers there are a number of large pyknotic nuclei. Very few round cells can be seen in this region. However, around this area and toward the periphery the muscle consists of degenerated remnants which are multinucleated and everywhere surrounded or invaded by lymphocytes. Here the lymphocytic reaction is marked. Lymph vessels filled with lymphocytes, as well as numerous small blood vessels, are found between many of the muscle remnants. Peripherally, a number of nuclei with very little cytoplasm about them lie among the round cells. The nuclei are, as a rule, not as large or as distinctly vesicular as those in the autotransplant, although some proliferation is apparently taking place. In addition to the lymphocytes, connective tissue cells are rather numerous in this area.

Fifteen-Day Autotransplant: There are present in this transplant, well formed slender muscle fibers with distinct and regular cross-striations. Very little degeneration of the fibers is seen. The muscle nuclei are still increased in number, but are not as numerous as in the earlier transplants; also they are in general smaller, more elongated and situated nearer the periphery of the fibers than the nuclei of earlier muscle pieces. There is almost no lymphocytic invasion, while a few very small blood vessels are seen between some of the fibers. Thus, the muscle now consists almost entirely of long slender fibers with good cross-striations staining normally, and showing still an increase of muscle nuclei. Nowhere can there be seen a true, new formation of muscle fibers with an outgrowth into an area which had originally been free of muscle tissue.

Fifteen-Day Homoiotransplant: The transplant is almost completely degenerated; it is surrounded by a connective tissue capsule

containing numerous round cells. Near the capsule, a few muscle remnants containing several peripherally placed nuclei are present, but centrally almost no muscle tissue is seen, it being replaced chiefly by blood vessels and a small number of round cells and connective tissue. However, some regeneration does take place, as is indicated by the appearance near the periphery of a very few small slender muscle fibers which are multinucleated, the nuclei being situated chiefly at the ends or the periphery of the fibers.

Twenty-Day Autotransplant: The muscle here has practically the same appearance as in the fifteen-day autotransplant. The fibers are well formed and slender. They also stain well and have distinct and regular cross-striations. They appear a little more mature than at fifteen days, the nuclei being less numerous, peripherally placed, spindle-shaped and more darkly staining. Small capillaries again are seen between the fibers while very few lymphocytes are present in the muscle. There is some sign of degeneration in a few areas at the periphery of the transplant where the fibers stain paler, appear granular and have no distinct, regular cross-striations.

Twenty-Day Homoiotransplant: In this piece the muscle fibers which occur along the side of the transplanted cartilage have been almost completely destroyed and replaced by round cells, connective tissue cells and small blood vessels. Only in a few places an occasional muscle remnant containing a few pyknotic nuclei is seen. Extending peripherally from the area first described is a group of large thick degenerated muscle fibers which are surrounded by a ring of round cells, connective tissue cells and blood vessels, together with occasional small degenerated muscle remnants. These large muscle fibers contain only a small number of round cells and appear to be the remains of the original transplanted muscle.

Twenty-Eight-Day Autotransplant: The muscle fibers as a rule have cross-striations and are rather long and slender. The nuclei are small, are not increased in number and are situated at the periphery. In some places, especially at the periphery of the transplant, the muscle tissue is degenerated, the fibers losing their stain and appearing granular and fibrillar. A very few of them are completely surrounded by connective tissue and are necrotic. Small blood vessels are scattered throughout the muscle tissue. No lymphocytic invasion of the muscle can be seen and while connective tissue is present about all of the muscle tissue, it does not invade the fibers. There are practically no lymphocytes in the connective tissue.

On the whole, the muscle fibers although somewhat irregular and degenerated in a few places seem to be mature and in good condition.

Thirty-Two-Day Homoiotransplant: To one side of the cartilage, there is a very dense area of lymphocytes which have almost completely destroyed the transplanted muscle in this area. The round cell reaction is extremely intense. In addition lymph vessels are packed with lymphocytes. In the middle of this area of round cells, and also approaching one end of the cartilage are seen rather numerous, very small and slender pale pink-staining muscle fibers which are spindle-shaped and which contain multiple, elongated nuclei at their periphery. Well defined cross-striations are present in a number of these fibers, which are well preserved in spite of the intense lymphocytic invasion about them. In one place in the periphery may be seen a number of small degenerated muscle remnants among the round cells. In this specimen, thus, the round cell reaction has become intense and has destroyed the greater part of the muscle; however, there is, even here, still a small amount of regeneration taking place.

Forty-Day Autotransplant: At one end of the cartilage transplanted muscle tissue is found. The fibers, on the whole, appear normal, stain well and have cross-striations. In the periphery a few of them are granular or show a fibrillar structure. The muscle nuclei are slender, elongated, stain darkly and are not increased in number. There is no lymphocytic invasion between or in the muscle fibers.

Forty-Day Homoiotransplant: The appearance of this transplant is very similar to that of the thirty-two-day homoiotransplant. The lymphocytic reaction is intense and masses of lymphocytes have replaced almost completely the transplanted muscle. In one area, at the periphery of the transplant, a number of muscle remnants are being invaded by round cells and connective tissue cells. In another area, likewise in the periphery, there are present a small number of short slender multinucleated muscle spindles, indicating that some regeneration is still taking place. However, no cross-striations are present in these spindles, which thus differ from those seen in the thirty-two-day homoiotransplant; here also, round cells and small blood vessels are invading and destroying the muscle spindles.

Fifty-Day Autotransplant: The transplanted muscle is represented

by a number of slender pink-staining fibers, which have distinct and regular cross-striations. The muscle nuclei are somewhat larger than normal, are multiple and situated at the periphery of the fibers. The muscle appears normal in every respect, except that the fibers are comparatively small and slender and contain an increased number of rather large nuclei. Fibroblasts are numerous and are invading and destroying many of the muscle fibers. Practically no lymphocytes are present.

Fifty-Day Homoiotransplant: On one side of the cartilage is an area containing many lymphocytes, fibroblasts and a few small blood vessels, which almost completely replace the transplanted muscle tissue. Occasionally, small, oval, pale pink muscle remnants which sometimes contain a few nuclei, are seen. These are the only traces of muscle tissue which remain. No evidence of muscle regeneration is seen.

Seventy-Day Autotransplant: The muscle fibers as a rule are small, but appear to be living. Many of them are somewhat irregular in outline. There is no lymphocytic invasion of the muscle; fat tissue is present between a number of the fibers.

Seventy-Day Homoiotransplant: The lymphocytic reaction in this piece is extremely intense, the lymphocytes taking up practically the whole of the transplant. Very few traces of muscle fibers remain. Occasionally some muscle nuclei lying within very small granular muscle remnants are seen, grouped together among the lymphocytes. Fibroblasts are rather numerous.

Eighty-Six-Day Autotransplant: The transplanted muscle has practically the same appearance as that of the seventy-day autotransplant.

Eighty-Six-Day Homoiotransplant: At one side of the cartilage there is an area of connective tissue in which there are several dense collections of lymphocytes; occasionally there is a lymph vessel full of lymphocytes. The round cells have replaced about all of the transplanted muscle. However, a few vesicular muscle nuclei are seen within very small slender spindle-shaped fibers which are lying among the lymphocytes. In some places a few degenerated fibrillar muscle remnants having no cross-striations are present.

One-Hundred-and-Eighteen-Day Autotransplant: The transplanted muscle fibers are living and well preserved at this stage. The large majority of them stain well and have well defined, regular, cross-

striations. They are of normal thickness, and appear in almost every respect like normal, mature muscle tissue. Some of the fibers, however, contain small chains of nuclei varying in number from three to eight, situated usually near the periphery. In some places, especially in the periphery, the fibers are small and granular and often show some invasion by a few connective tissue cells and lymphocytes. Small blood vessels are rather numerous in this region. The connective tissue cells and, to a less extent, the lymphocytes are somewhat increased in this autotransplant. In several one-hundred-and-eighteen-day autotransplants, no muscle tissue could be found, this being possibly due to the fact that the part of the piece containing muscle was not obtained for examination.

One-Hundred-and-Eighteen-Day Homoiotransplant: Almost no muscle tissue can be seen in this transplant, although at one end a very few small degenerated remnants are still present among the lymphocytes, which are very numerous in this region and have replaced the muscle. In other places, only connective tissue containing a few small blood vessels and scattered lymphocytes is observed.

Conclusions

In the autotransplants, during the first few days following the transplantation, the muscle is invaded by polymorphonuclear leucocytes and the greater part of it becomes necrotic. However, as early as four days after the operation there is found some proliferation of muscle nuclei within preserved fibers. At six days, we note a marked proliferation of muscle nuclei with the formation of long slender muscle spindles, many of which are again developing cross-striations. The number of leucocytes is decreased. The spindles subsequently take on a more mature appearance, the cross-striations becoming more definite and numerous, the nuclei decreasing in number and assuming a more nearly peripheral situation. At fifteen days the muscle consists chiefly of long slender fibers which contain good cross-striations, but which still show an increase in the number of muscle nuclei. Lymphocytes, which at earlier periods were present in small numbers, have at this time almost completely disappeared. At twenty-eight days, the muscle, except for slight signs of degeneration in a few areas, appears mature and normal in almost every respect. This is also the case at forty days, the muscle having reached

its greatest development at this stage. At fifty days there is noticeable much fibroblastic invasion of the muscle fibers, which are comparatively small, contain an increased number of nuclei but which have definite, regular cross-striations. At seventy and eighty-six days the muscle is still living and also at one hundred and eighteen days, at which period the fibers are in very good condition and look normal, except that in some of them there are a few small chains of nuclei and that there is also some increase in the connective tissue cells and lymphocytes. It should be mentioned, however, that not in all experiments was muscle seen at one hundred and eighteen days.

In the homoiotransplants at two and four days, the appearance of the muscle is similar to that of the autotransplants, but almost no evidence of nuclear proliferation is present in the four-day transplant, in contrast to the corresponding autotransplant. At six days the lymphocytic reaction is becoming distinct, although it is not yet very marked. The degree of regeneration, as shown by the number and appearance of the muscle nuclei, as well as of the spindles, is less in the homoiotransplant. At ten days the lymphocytic reaction increases in intensity and the muscle fibers undergo degeneration, with the consequent formation of many muscle remnants. There is still a slight proliferation of muscle nuclei at this stage. The lymphocytic invasion of the muscle and its destruction increase. At thirty-two days the lymphocytic reaction has become so intense that the muscle has been almost completely replaced by such collections of small round cells. However, among the round cells there can be seen a few small multinucleated muscle spindles with well marked cross-striations, indicating that slight regeneration may still be proceeding or that regenerated cells are surviving. The lymphocytic invasion continues to increase, with connective tissue cells also playing a part, and at fifty days only a few muscle remnants remain. At seventy days the round cell reaction has reached its height and now, as well as somewhat later, only a very few degenerated muscle remnants remain. Finally, at one hundred and eighteen days, practically no muscle tissue can be found, it having been replaced by lymphocytes, connective tissue and a few small blood vessels. Thus, it is seen that in the homoiotransplants regeneration takes place in a similar manner to the autotransplants; but the regeneration is less intense. Furthermore,

the degenerative processes due to the lymphocytes and, to a less extent, to the connective tissue, are much more pronounced in the homoiotransplants.

The differences between auto- and homoiotransplantation of cross-striated muscle tissue described in our experiments are in accordance with the results obtained by Loeb and his co-workers in the case of other tissues and also, in a general way, with the subsequent work of Shinya in the case of cross-striated muscle. However, Shinya, as has previously been mentioned, assumes that there exists a difference in the manner of regeneration between auto- and homoiotransplants, inasmuch as, in the former, not only muscle spindles develop, but also independent myoblasts appear at the edge of the wound, whereas in the latter only muscle spindles were seen. No such differences between these two types of transplants were seen in our experiments. Furthermore, in Shinya's experiments the muscle tissue of the autotransplants was replaced by connective tissue as early as thirty-five days after operation, while in our experiments good transplanted living muscle tissue was found as late as one hundred and eighteen days after transplantation, this being the longest period at which autotransplants were examined. In the homoiotransplants, Shinya found complete necrosis at twenty-seven days, while in our experiments a few multinucleated muscle spindles were present as late as forty days. Saltykow carried out only autotransplantations of cross-striated muscle and his description of the latter process is similar to ours; however, in contrast to our findings, he reported practically a complete disappearance of the cross-striated muscle fibers at the beginning of the fourth month.

Our experiments prove that after autotransplantation striated muscle can survive in the host at least for a period of four months without functioning. How much longer autotransplanted muscle could thus be preserved in the rat, we do not know. In certain places we found some indication that there was an increased activity of fibroblasts among the transplanted muscle fibers in the later periods. This may be taken as an indication that even in autotransplants some destruction takes place in the course of time.

As to the homoiotransplants, their injury and destruction are evidently due to two factors. Primarily, the regeneration and perhaps also the preservation in the first period following transplanta-

tion, are less in the homoiotransplants than in the autotransplants. This indicates a direct injury of the transplant by homoiotoxins, a conclusion which is in accordance with the observations of Hesselberg [10] and Loeb in the case of the unstriated muscle tissue of the uterus; and of Loeb in the case of the placentoma formation in the transplanted uterine tissue. In the second place, the lymphocytes and also the connective tissue take an active part in the destruction of the homoiotransplanted muscle tissue while they leave intact the autotransplanted muscle. In this respect striated muscle tissue behaves, after transplantation, like all the other tissues so far transplanted into other individuals of the same species.

There remains still one point to be discussed; namely, the evident lack of extensive regeneration, if we use this term in its strict sense, as indicating an outgrowth of tissue into a defect, leading to a filling in of the defect. As far as we can make out such an outgrowth does not take place to any noticeable extent; instead, we find a reformation, through growth processes, of a part of the degenerated muscle. We have, therefore, to deal in the case of transplantation of striated muscle tissue with a process in some respects akin to that which we find in compensatory hypertrophy, as for instance, in the liver, rather than with a regeneration such as takes place in the case of the epidermis.

Summary

1. After autotransplantation of striated muscle tissue in the rat, well preserved muscle tissue was found as late as one hundred and eighteen days after transplantation, the further fate of the muscle tissue not having been studied yet.

2. After homoiotransplantation, there is less regeneration of the muscle tissue in the first period after transplantation, and much more degeneration due to invasion by lymphocytes and, to a less extent, by connective tissue at subsequent periods. As a result of these conditions, homoiotransplanted muscle disappears much earlier than autotransplanted muscle, no preserved tissue being present at fifty days.

It is therefore probable that in addition to the action of the lymphocytes, the homoiotoxins may injure the muscle tissue directly, diminishing the regenerative process and increasing the subsequent degeneration.

3. Nowhere was there seen a true outgrowth of muscle fibers, either in continuity with the preformed transplanted muscle or by budding. The old muscle tissue, after a primary degeneration of its major portion, undergoes regenerative processes, consisting in an increase of its sarcoplasm and a proliferation of its nuclei; subsequently there is a differentiation of the sarcoplasm into typical striated muscle, in which cross-striations are again formed. In the course of time the nuclear proliferation becomes less active; the nuclei decrease in number and become situated peripherally within the fibers, while the fibers as such increase in size, until finally there are again present almost normal muscle fibers. This growth process is, in certain respects, comparable to compensatory hypertrophy or hyperplasia rather than to regeneration in the strict sense.

REFERENCES

1. Lexer, E. Die freien Transplantationen. Stuttgart, 1919.
2. Saltykow, S. *Arch. f. Entwicklngsmechn. d. Organ.*, 1900, ix, 329.
3. Askanazy, M. *Virchows Arch. f. path. Anat.*, 1891, cxxv, 520; *Wien. med. Wchnschr.*, 1912, lxii, 27, 129.
4. Jores–Köln, L. *Verhandl. d. deutsch. path. Gesellsch.*, 1909, xiii, 103.
5. Shinya, S. *Beitr. z. path. Anat. u. z. allg. Pathol.*, 1914, lix, 132.
6. Loeb, Leo. *J. A. M. A.*, 1915, lxiv, 726; *Biol. Bull.*, 1921, xl, 143.
7. Myer, M. W. *Arch. f. Entwicklngsmechn.*, 1913, xxxviii, 1.
8. Sale, L. *Arch. f. Entwicklngsmechn.*, 1913, xxxvii, 248.
9. Krause. Habilitationschrift. Halle, 1878.
10. Hesselberg, Cora. *J. Exper. Med.*, 1915, xxi, 164.